A STUDY ON SECONDARY BACTERIAL INFECTIONS IN PATIENTS WITH VENOUS LEG ULCER AND THEIR ANTIMICROBIAL SUSCEPTIBILITY PATTERN IN A TERTIARY CARE HOSPITAL

Dissertation submitted to

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In partial fulfillment of the regulations

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M.D. (MICROBIOLOGY)

BRANCH – IV

MADRAS MEDICAL COLLEGE

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APRIL 2016

CERTIFICATE

This is to certify that this dissertation titled "**A STUDY ON SECONDARY BACTERIAL INFECTIONS IN PATIENTS WITH VENOUS LEG ULCER AND THEIR ANTIMICROBIAL SUSCEPTIBILITY PATTERN IN A TERTIARY CARE HOSPITAL"** is a bonafide record of work done by **DR. G.BANUMATHI RATHIKA,** during the period of her Post Graduate study from 2013 to 2016 under guidance and supervision in the Institute of Microbiology, Madras Medical College and Rajiv Gandhi Government General Hospital, Chennai- 600003, in partial fulfillment of the requirement of **M.D MICROBIOLOGY** degree Examination of **The Tamilnadu Dr. M.G.R Medical University to be held in April 2016.**

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I declare that the dissertation entitled **"A STUDY ON SECONDARY BACTERIAL INFECTIONS IN PATIENTS WITH VENOUS LEG ULCER AND THEIR ANTIMICROBIAL SUSCEPTIBILITY PATTERN IN A TERTIARY CARE HOSPITAL**" submitted by me for the degree of M.D. is the record workcarried out by me during the period of **OCTOBER 2014–AUGUST 2015** under the guidance of **Dr. S.Vasanthi, M.D.,** Professor, Institute of Microbiology, Madras Medical College, Chennai. This dissertation is submitted to The **Tamil Nadu Dr.M.G.R. Medical University, Chennai,** in partial fulfillment of the University regulations for the award of degree of M.D., Branch IV (Microbiology) examination to be held in April 2016.

Place: Chennai **Signature of the candidate**

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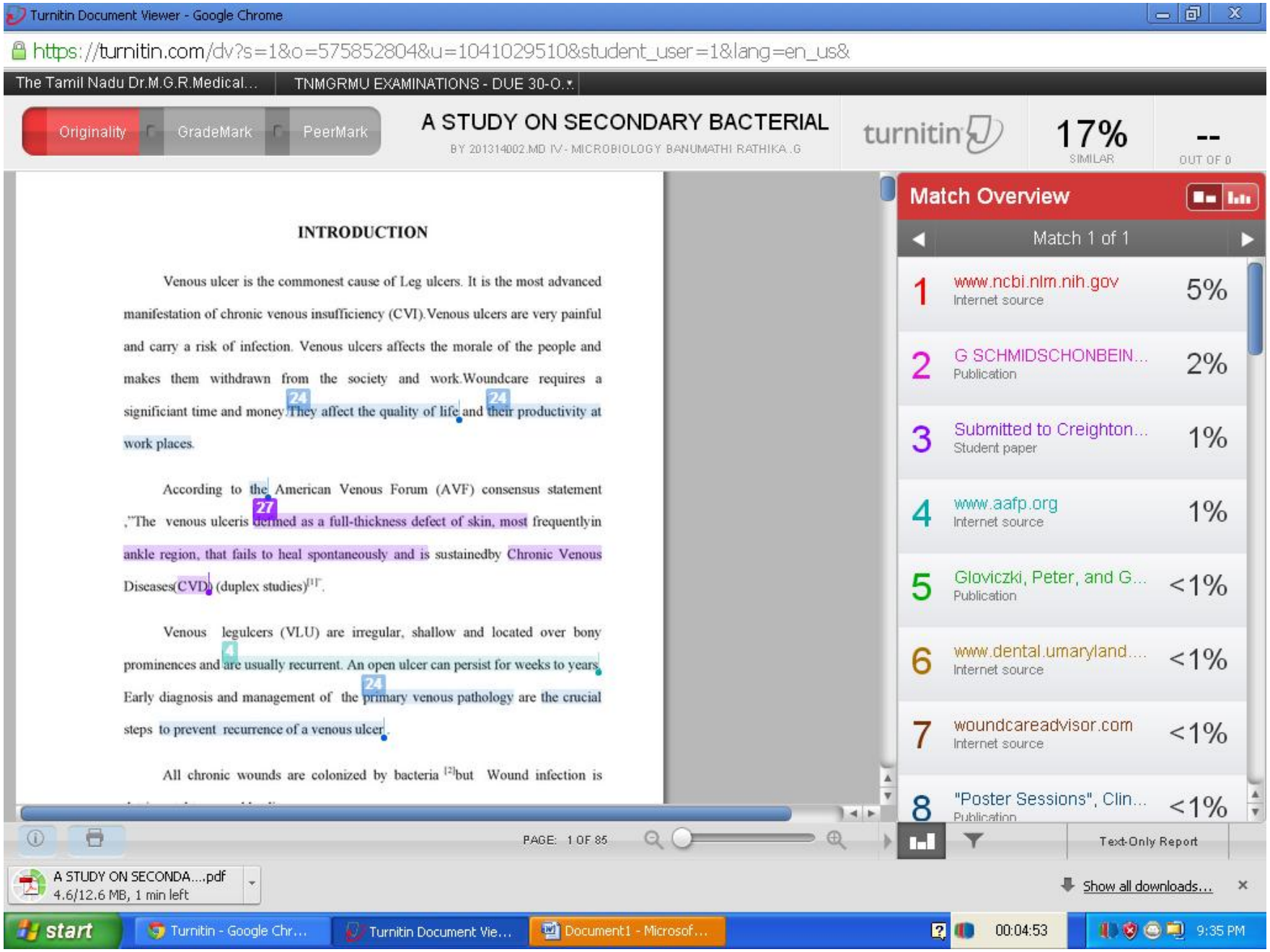
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INTRODUCTION
Venous ulcer is the commonest cause of Leg ulcers. It is the most advanced manifestation of chronic venous insufficiency (CVI).Venous ulcers are very painful and carry a risk of infection. Venous ulcers affects the morale of the people and makes them withdrawn from the society and work.Woundcare requires a significiant time and money.They affect the quality of life and their productivity at work places.
According to the American Venous Forum (AVF) consensus statement ,"The venous ulceris defined as a full-thickness defect of skin, most frequentlyin ankle region, that fails to heal spontaneously and is sustainedby Chronic Venous Diseases(CVD) (duplex studies)[1].
Venous legulcers (VLU) are irregular, shallow and located over bony prominences and are usually recurrent. An open ulcer can persist for weeks to years. Early diagnosis and management of the primary venous pathology are the crucial steps to prevent recurrence of a venous ulcer.
All chronic wounds are colonized by bacteria [2]but Wound infection is
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ABSTRACT

A STUDY ON SECONDARY BACTERIAL INFECTIONS IN PATIENTS WITH VENOUS LEG ULCER AND THEIR ANTIMICROBIAL SUSCEPTIBILITY PATTERN IN A TERTIARY CARE HOSPITAL.

INTRODUCTION

Venous legulcer is the commonest cause of Leg ulcers. It is the most advanced manifestation of chronic venous insufficiency (CVI).Venous ulcers are very painful and carry a risk of infection. Venous ulcers affect the morale of the people and make them withdrawn from the society and work.Wound Care requires a significiant time and money.They affect the quality of life and their productivity at work places.

The venous ulcer is defined as a full-thickness defect of skin, most frequently in ankle region, that fails to heal spontaneously and is sustained by Chronic Venous Diseases(CVD) (duplex studies).

MATERIALS AND METHODS

This is a cross sectional study

Study period : October 2014 to August 2015.

Study population : 100 patients with venous leg ulcers**.**

The two bits of tissue are collected from the venous ulcers under aseptic precautions and processed quantitatively. Anaerobes are identified using Anaerobic identification disks. Antibiogram was done using Kirby-Bauer disk diffusion method for aerobes.

RESULTS

Out of 100 ulcers,46% of the ulcers were ulcers with single organism. 45% of the ulcers were polymicrobial.Out of 100 Ulcers,48% ulcers were found to be infected.56 pathogens were isolated from these ulcers.Staphylococcus aureus was the commonest pathogen to be isolated.Methicillin resistant Staphylococcus aureus was isolated from 13% of the ulcers.38.4% of MRSA were moderate biofilm producers. Streptococcus pyogenes were isolated from 3% of the cases. ESBL producers were 18.3% among the Enterobactericeae.

CONCLUSION

The quantitative culture of tissue in venous leg ulcers helps to assess the bacterial load in the ulcers and aids in treatment modalities.

KEY WORDS

Venous leg ulcers,Quantitative culture of tissue, MRSA, Chronic venous insufficiency

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INTRODUCTION

Venous ulcer is the commonest cause of Leg ulcers. It is the most advanced manifestation of chronic venous insufficiency (CVI).Venous ulcers are very painful and carry a risk of infection. Venous ulcers affects the morale of the people and makes them withdrawn from the society and work. Wound care requires a significant time and money. They affect the quality of life and their productivity at work places.

According to the American Venous Forum (AVF) consensus statement, "The venous ulcer is defined as a full-thickness defect of skin, most frequently in ankle region, that fails to heal spontaneously and is sustained by Chronic Venous Diseases(CVD) (duplex studies)[1]".

Venous leg ulcers (VLU) are irregular, shallow and located over bony prominences and are usually recurrent. An open ulcer can persist for weeks to years. Early diagnosis and management of the primary venous pathology are the crucial steps to prevent recurrence of a venous ulcer .

All chronic wounds are colonized by bacteria [2] but Wound infection is detrimental to wound healing.

The constant presence of bacteria in the venous ulcers stimulates the host immune defenses leading to the production of inflammatory mediators. Cytotoxic enzymes and free oxygen radicals are continuously released as neutrophils keep

migrating into the ulcer. Thrombosis and vasoconstrictive metabolites cause wound hypoxia, leading to enhanced bacterial proliferation and continued tissue damage[3].Evasion of the body's immune system by bacteria make it difficult to be negated by the host defenses. This development of "immune tolerance" can mask the infection and may prevent treating the infection.

Biofilm formation is common in chronic wounds because of the moist adherent environment where bacteria aggregate and become embedded in a self-secreted exopolysaccharide matrix. The presence of such biofilms results in inefficient eradication of bacteria by antibiotic treatment and host defense mechanisms.[4]It delays wound healing and favors the emergence of resistant bacteria.

The interpretation of clinical findings and microbiological investigations in patients with chronic leg ulcers from colonization to infection can help clinicians with the management of ulcers. The quantitative wound culture will help to detect bacterial burden.

This study is done to determine the etiological agents infecting and colonizing the venous leg ulcers and their antimicrobial sensitivity pattern. This study will be also useful for distinguishing the patients with infected ulcers from colonized one,therefore preventing inadvertent use of antibiotics and restricting the use only to the appropriate infected population.

AIMS

1. To determine the bacteriological profile in venous ulcer patients
2. To categorize the wound based on bacterial burden
3. To study the resistance pattern of the isolates

OBJECTIVES

1. To isolate and identify the bacteria infecting the patients with venous leg ulcers.
2. To quantify the bacterial burden of the wound .
3. To determine the antimicrobial susceptibility pattern of the isolated organisms and study their resistance pattern in venous ulcers

REVIEW OF LITERATURE

Venous ulceration is the most common and serious consequence of severe chronic venous insufficiency. They are the most common cause of leg ulcers, accounting for 60-80% of them. The prevalence of VLUs is between 0.18% and 1%.Over the age of 65, the prevalence increases to 4%[5].The ulcers which persist for more than 6 weeks are defined as chronic VLUs[6]

DEFINITIONS

THE AVF CONSENSUS STATEMENT[1]: Venous ulcer is defined as a full-thickness defect of skin, most frequently in ankle region, that fails to heal spontaneously and is sustained by CVD (duplex studies).

THE SCOTTISH GUIDELINE DEFINITION [6]: Chronic venous leg ulcer is defined as an open lesion between the knee and the ankle joint that remains unhealed for at least four weeks and occurs in the presence of venous disease. Studies reviewed in this guideline included patients with venous leg ulcers, irrespective of the method of diagnosis of venous reflux.

FRENCH HEALTHCARE SYSTEM GUIDELINES [7]:

A pure venous ulcer is defined, by professional agreement, as a leg lesion, which has not healed within a month (except in cases of recurrent ulcers when a diagnosiscan be made in less than a month); with a pathophysiology due to ambulatory venous hypertension, which maybe secondary to: reflux in superficial,

perforating or deep veins, and/or obstruction of the deep veins, and/or calf muscle pump dysfunction; where there is no arterial involvement.

HISTORY

The word “Varicose” is derived from Greek, meaning “Grape like”. Hippocrates was the first person to appreciate the relationship between calf pump dysfunction and venous ulceration .[8]

In the Susrutha Samhitha, Susrutha had described superficial thrombophlebitis and its treatment by *Jalaukavacharan* or blood letting using leech.[9]

Aurelius Cornelius Celsus (53 BC to 7 AD) used bandages for the treatment of leg ulcers. Galenus (130–200 AD) excised segments of veins controlled between ligatures.[9]

The theory of valvular incompetence as a cause for varicose veins was put forward by Hieronymus Fabricius in 1603. Dilatation of vein as a cause for valvular incompetence was suggested by Richard Wiseman (1676). He coined the term varicose ulcer[9].

Interestingly, the management of venous ulcers has not progressed very far beyond that advocated by John Hunter over 200 years ago. Hunter said, “The sores of poor people are often mended by rest, a horizontal position, fresh provisions, and warmth.”

John Gay(1868), a London surgeon, described the relationship of venous ulceration to ankle perforating veins.He identified the importance of deep vein thrombosis (DVT) and the importance of ankle perforating vein in the genesis of leg ulcers. He coined the term "V**enous ulcer"**[9]

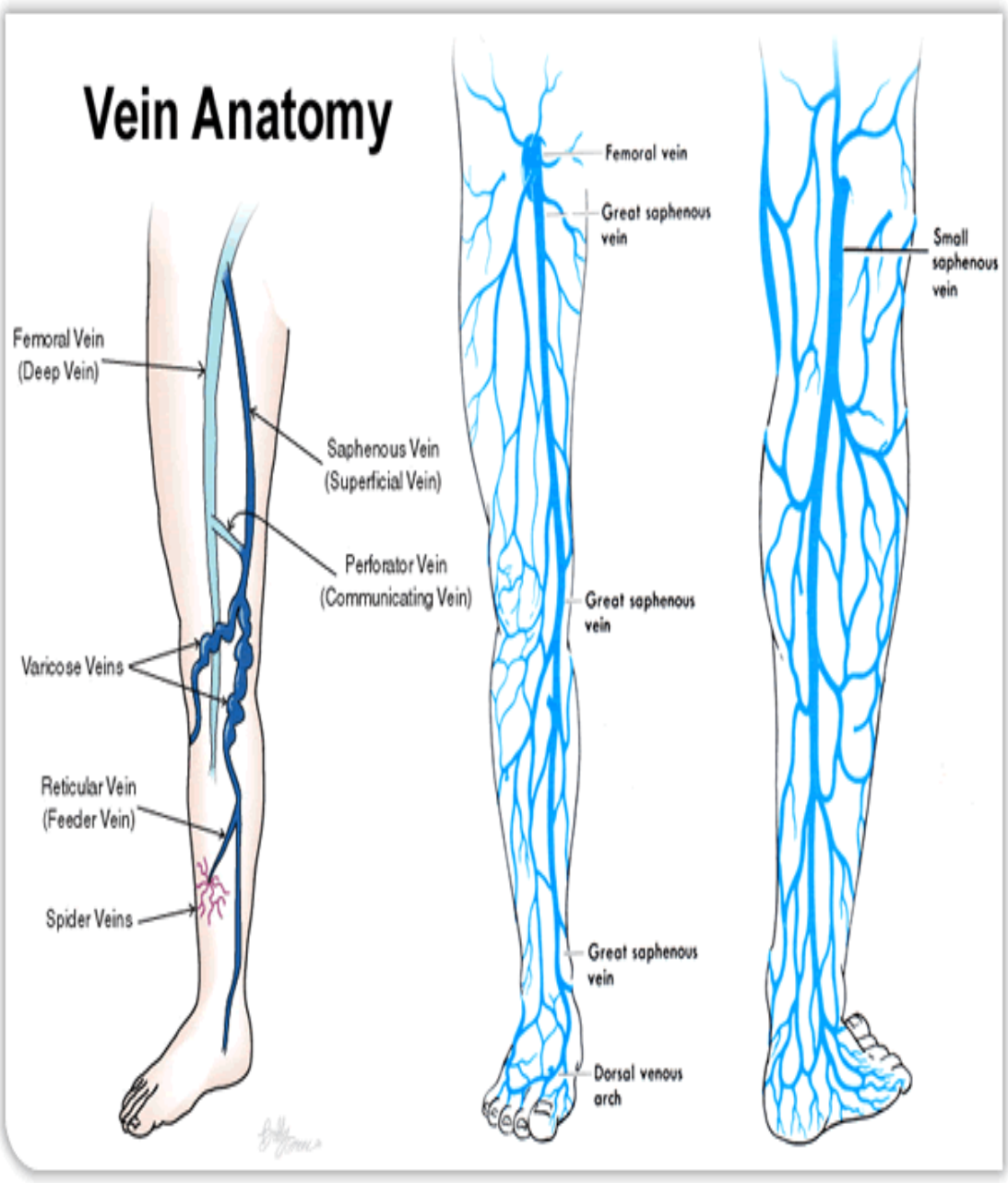
Sir Benjamin Brodie (1846) described a bedside test for the identification of incompetent valves, by the use of constriction and palpation of the limb. Friedrich Trendelenburg (1844 – 1924) refined the method in 1890.

An important breakthrough was by Paul Gerson Unna (1896). He introduced "The Unna Boot". He incorporated emollient compounds in a dressing that became increasingly rigid He was responsible for developing dermatology as an independent speciality[10]

Conrad Jobst (1930), a successful engineer, suffered from refractory venous ulcers. Jobst introduced graduated compression stocking for control of his own disease.[9]

Phlebography was introduced by Berberich and Hirsch, Sicard and Forestier in the 1920s. Dos Santos in 1938 described ascending venography for the diagnosis of DVT. by Sigel and Colleagues (1967) introduced the Doppler for the evaluation of venous diseases. The present gold standard for the assessment of vascular disorder is Duplex ultrasound .It was introduced by Szendro, Nicolaides, Myers, Malouf et al. in 1986[9].

FIGURE 1: ANATOMY OF VENOUS SYSTEM OF LOWER LIMB



Several academic forums are actively involved in the study and research on venous diseases[9]. They are

The American Venous Forum (AVF) 1987

The European Venous Forum 2000

Two academic societies in India that are active in the field of venous diseases are

- Vascular Society of India (VSI): 1994
- Venous Association of India (VAI): established in 2007 to promote research and study of venous diseases.

The society maintains healthy collaboration with other sister organizations across the world.

ANATOMY OF THE VENOUS SYSTEM OF LOWER LIMB[11]

The lower extremity venous system includes

1. The superficial,
2. The Deep, and
3. The Perforating veins

The antegrade flow of blood within these veins is ensured by a system of muscular venous pumps and bicuspid valves

The Superficial Veins

The superficial venous system includes [11]

The reticular veins

The great saphenous vein and their tributaries

The small saphenous veins

The reticular veins

The reticular veins are a network of veins running parallel to the skin surface and lying between the saphenous fascia and dermis. They drain the lower extremity skin and subcutaneous tissue.[11] These veins communicate with either saphenous tributaries or the deep veins through perforators.

THE GREAT SAPHENOUS VEIN (GSV)

It is the longest vein in the body, situated in the Superficial fascia and is seen easily through the skin .The Great saphenous vein is formed on the medial aspect of dorsum of foot by the union of dorsal venous arch and digital vein from the medial great toe.The main trunk of the great saphenous vein has six valves.It has nine main tributaries among which the posterior arch vein drains a network of medial ankle veins and is important in that the posterior tibial perforators join this vein rather than the main trunk of the great saphenous vein.

THE SMALL SAPHENOUS VEIN (SSV)

The small saphenous vein, formerly known as the short or lesser saphenous vein, arises from the dorsal pedal arch and ascends posterolaterally from behind the lateral malleolus to terminate in the popliteal vein. The small saphenous vein usually has 7 to 10 closely spaced valves.The lateral arch vein , the major tributary of the small saphenous vein communicates with the peroneal vein through the lateral calf perforators. The small saphenous vein communicates with the medial ankle perforators through several tributaries.

THE DEEP VEINS

The major deep veins of the lower extremity follow the course of the associated arteries and, with the exception of the femoral vein, are named accordingly.The deep venous system of the calf includes the tibial, peroneal, soleal and gastrocnemial veins. The anterior tibial, posterior tibial and peroneal veins are the venae comitantes of the corresponding arteries.The paired veins communicate in a plexiform arrangement around the artery. [11]The number of deep venous valves increases from cranial to caudal.

THE PERFORATING VEINS

There are two types of Perforators.

1. Direct-Drain into Deep veins

2. Indirect-Drain into the venous sinuses of calf muscles

There are about 150 Perforating veins in the lower extremity[12].

The direct perforators are localized into five groups.

They are 7-9 cms,10-12 cms,18-22 cms,23-27 cms and 28-32 cms proximal to the medial malleolus.[11] The indirect perforators are randomly distributed. The medial calf perforators are important clinically.

Perforators are present in foot, ankle ,below knee ,around the knee. There are perforators of Femoral canal and Inguinal perforators. The foot perforators

directly flow towards the superficial veins. All others directly flow to the deep system. The major perforators of the medial calf and thigh have one to three valves that direct flow from the superficial to the deep veins.[11]

Venous Sinuses of the Calf Muscle[11]

These are large thin-walled blood filled spaces located mostly in the soleus (1–18 sinuses) and to a less extent in the gastrocnemius muscle. Venous sinuses of the soleus muscle drain into the posterior tibial vein through multiple large, short, and tortuous soleus veins. Gastrocnemius veins drain the two heads of the muscle and empty into the popliteal vein distal to the Saphaneopopliteal junction. These sinuses are filled from the superficial veins and the reticular venous plexuses. They can hold a large volume of blood and can function as chambers of the peripheral heart.

THE CALF MUSCLE PUMP

The accumulation of blood in the lower extremity veins while upright is limited by the physical properties of the venous wall, the function of the venous valves, and theaction of the calf muscle pump.Three muscle pumps are The foot, The calf, and The thigh muscle pumps.[11]

The action of these valved pumps is dependent on the deep fascia of the leg.It constrains the muscles during contraction and allows high pressures to be generated within the muscular compartments.With contraction of the calf, pressure in the posterior compartment rises to as high as 250 mmHg.The veins are emptied

of blood, and resting venous pressure is lowered as the valves prevent retrograde flow. The muscular venous sinuses are the principal collecting system of the calf muscle pump.The constituents of the Calf muscle pump are Soleus sinuses (one to eighteen) and gastrocnemial network .The soleal sinuses communicate with the posterior tibial vein in the proximal calf .The gastrocnemial network coalesces to form the paired gastrocnemial veins draining into the popliteal vein.[11]

PATHOPHYSIOLOGY[13]

PATHOPHYSIOLOGY OF CHRONIC VENOUS INSUFFICIENCY(CVI)

Blood returns from lower extremities against gravity to Inferior vena cava (IVC) through deep and superficial venous system located within muscles and deep fascia of legs. The superficial system consists of GSV and SSV located within Subcutaneous fat. Valves present within all these systems prevent retrograde flow of blood .A portion of blood from superficial systems is directed to deep system through communicating perforators. While standing, about 22% of total blood volume is localized in lower extremities.

Thegeneral pathophysiologyof chronic venous insufficiency are

1. Sustained central outflow obstruction following the thrombosis of central portions of venous tree
2. Congenital abnormality
3. Reflux in the deep venous thrombosis or a pressure volume overload associated with varicose veins
4. Congenital autosomal dominant absence of all venous valves (rare)

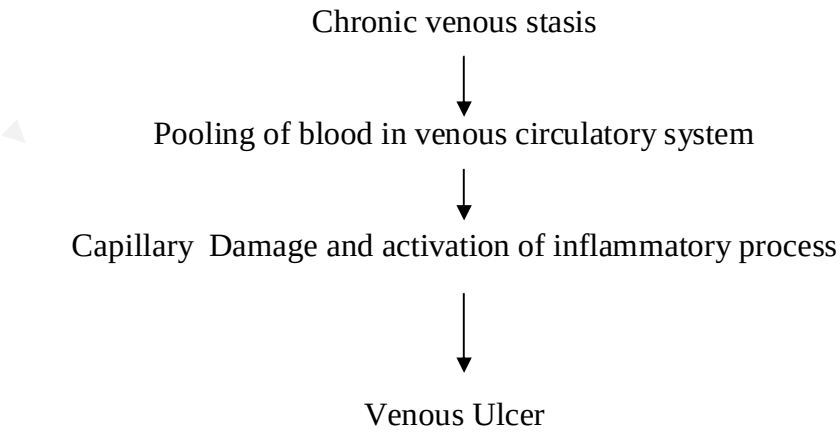
PATHOPHYSIOLOGY OF VENOUS ULCER[13]

The most common site of occurrence is the retromalleolar fossa located between the medial malleolus and Achilles tendon.Ulcers can occur at any site where venous hypertension is especially pronounced due to local circumstances.ie proximity to incompetent perforators and on lateral or medial dorsum of foot.

Venous incompetence and venous hypertension are thought to be the primary mechanisms for ulcer formation.

Factors that lead to venous incompetence are

1. Immobility
2. Ineffective pumping of the calf muscle
3. Venous valve dysfunction from trauma, congenital absence, venous thrombosis, or phlebitis [13].



Leukocyte activation, endothelial damage, platelet aggregation, and intracellular edema contribute to venous ulcer development and impaired wound healing.

Chronic venous disease is accompanied by infiltration of circulating leukocytes in the microcirculation that reduces local capillary perfusion .This enhances formation of free radical oxygen, delivery of proteolytic enzymes, synthesis and release of inflammatory molecules such as leukotrienes, prostaglandin, bradykinin and cytokines.This leads to tissue degradation. Vascular endothelial growth factor (VEGF), by definition, is a potent angiogenic factor which enhances endothelial permeability. Both VEGF expression and its receptor expression (Flk-1/KDR) are upregulated during the inflammatory reaction. Venous ulcer exudates inhibit the growth of human endothelial cells[13].

Transforming growth factor beta(TGF) is another cytokine whose expression is upregulated in patients with venous ulcers.It is related to tissue remodeling by stimulating the formation of the granulation tissue, proliferation of fibroblasts, and synthesis of collagen fibers. An important link between inflammation and skin changes may be by way of Ca/Zn-dependent endoproteinases (Matrix Metalloproteinases (MMPs)) and serine proteinases.[13]

Chronic Venous ulcers are characterized by excessive proteolytic activity, which degrades extracellular matrix and growth factors and their receptors. There is an increase of neutrophil elastase and lactoferrin from activated neutrophils in

patients under transient conditions of venous hypertension and with chronic venous insufficiency. Neutrophils and macrophages also release several MMPs. Venous leg ulcers have elevated expression of Extracellular MMP inducer (EMMPRIN; CD147) which increases the MMP expression [13].

Another factor that contributes to the lack of proper restructuring in venous ulcer fibroblasts demonstrates decreased proliferative responses to growth factor stimulation. Venous diseases have recurrent inflammation without definitive resolution. The inflammatory cascade remains active. Telangiectases develop into varicose veins, skin edema, pigmentation, venous eczema, and into active venous ulcers. Treatment needs to interfere with the inflammatory cascade that causes tissue damage but cannot be targeted against the tissue repair mechanism.

ROLE OF LYMPHATICS IN VENOUR ULCERS[14]

Lymphatic function is reduced in venous ulcer patients. In severe Chronic venous insufficiency and venous ulcers, Lymphatics become compromised. Oedema and skin changes occur. In severe CVI, lipodermatosclerosis may occur with ulceration.There is complete absence of lymphatics in ulcer bed and there is marked decrease of lymphatics surrounding the ulcer. The presence of edema complicates the management of CVI and particularly venous ulceration leading to poor wound healing.

PREDISPOSING FACTORS

The following factors account for chronic venous insufficiency and venous ulcers.[15]

1. Older age
2. Obesity
3. Varicose veins
4. Heart failure
5. Diabetes
6. Rheumatoid arthritis
7. Nephrosis
8. History of venous thrombosis
9. Multiple pregnancies
10. Hypertension
11. Life style and Family History
12. Occupation

About 5% of patients will develop leg ulcers following a clinically apparent DVT .

Congenital vein abnormalities-, Klippel-Trenauay-Parkes-Webber syndrome[16]is present at birth .Older children have active to healed venous ulcer. Rheumatoid arthritis, systemic vasculitis adversely affect the prognosis and the outcome of the treatment[17]

CLINICAL FEATURES

Patients with ulcer due to Chronic venous insufficiency complain of lower extremity pain and swelling of the leg usually beginning near the medial malleolus or gaiter region. CVI causes circulatory dysfunction on the macro- and microvascular level leading to a variety of clinical manifestations like lower extremity edema due to fluid accumulation in the dependent lower leg, pain, dilated veins, and skin changes.

Symptoms are worse at the end of the day, exacerbated when the leg is dependent and relieved by leg elevation in patients with the history of recurrent cellulitis ,DVT or previous superficial venous surgery.The prominent clinical features that indicate infection include

Fever,

Increased pain,

Increased swelling ,

Discharge from ulcer,

Foul smell

The Clinical-Etiology-Anatomy-Pathophysiology (CEAP) classification was developed to standardize the classification of patients with CVI. It was introduced in 1994 and revised in 2004.[1]according to AVF's recommendations,all Venous leg ulcers are evaluated with the CEAP score.

Anatomic classification is based on the involvement of the deep, superficial, or perforating veins while the pathophysiological classification describes the underlying mechanism of CVI as obstruction, valvular incompetence, or a combination of both conditions.

Clinical classification of CVI[1]

C0: no visible or palpable signs of venous disease

C1: telangiectasies or reticular veins

C2: varicose veins

C3: edema

C4a: pigmentation or eczema

C4b: lipodermatosclerosis or atrophie blanche

C5: healed venous ulcer

C6: active venous ulcer

Etiologic classification

Ec: congenital

Ep: primary

Es: secondary (post-thrombotic)

En: no venous cause identified

Anatomic classification

As: superficial veins

Ap: perforator veins

Ad: deep veins

Pathophysiologic classification

Pr: reflux

Po: obstruction

Pr,o: reflux and obstruction

Pn: no venous pathophysiology identifiable

Venous-Severity-Scoring[1]

The venous severity score (VSS) provides a more detailed assessment of CVI by assigning a numeric score to three components: clinical severity, anatomic segment, and disability[10]. VSS provide a more accurate tool for assessing a patient's response to treatment. It was designed to complement, not to replace CEAP.

In 2000 the AVF developed the three-part Venous Severity Score:

1. Venous Clinical Severity Score (VCSS) –revised in 2010,
2. Venous Segmental Disease Score (VSDS), and
3. Venous Disability Score (VDS) - a modification of the original CEAP disability score.

DIAGNOSIS

HISTORY AND CLINICAL EXAMINATION[1]:

Clinical history :Duration,recurrence,pain,trauma, and other co morbid factors are considered. Any clinical symptoms of infection,odour are also taken into consideration.

Physical examination of leg ulcer are evaluated. Examination of both legs are done.palpation of peripheral pulses, edema if present whether it is pitting or nonpitting type are done.

Signs of venous hypertension such as varicose veins, hemosiderin pigmentation, varicose eczema, atrophie blanche (Healed venous ulcer) and lipodermatosclerosis are noted.[17]

Range of movements for knee ,ankle and Hip are also determined.

Clinical assessment of ulcer includes the

a. The site of the ulcer- Locationof the ulcer: Anterior to medial malleolus, pretibial area or lower third of leg (gaiter region) gives clue to the underlying cause of ulcer.

b. Size and depth,

c. The edge and margins,

d. Thefloor,and base,

e. Condition of the surrounding skin.

f. Ankle/brachial pressure index (ABPI): It is an objective evidence to substantiate the presence or absence of significant peripheral arterial diseases. It is the ratio of the ankle to brachial systolic pressure It is measured either using a sphygmomanometer or hand held Doppler device.ABPI 0.8 -1.2 indicates good arterial supply and these patients can be given compression therapy safely.[5]

VASCULAR ASSESSMENT[1]

Doppler measurement of ankle/brachial pressure index- To indicate any arterial insufficiency

Duplex ultrasound-This investigation is done to reveal any obstruction in veins and arteries.Blood flow through Valves, superficial and deep veins can be visualized directly.

Photoplethysmography-measures the venous refill time. Refill time is abnormally increased in patients with venous diseases (<25 seconds).It also determines the efficiency of calf muscle Pump.[18]

Pulse oximetry-It measures the red and infrared light absorption of oxygenated and deoxygenated blood .Oxygenated blood absorbs more red light and deoxygenated blood more infrared light. Pulse oximetry is considered to support the diagnosis of venous ulcer.

Toe brachial pressure index (TBPI)-Non invasive test to measure the arterial perfusion in toes. It is used in diabetics and renal disease.

MICROBIOLOGY

Skin is the mechanical barrior to microorganisms .The normal flora and pH controls the invasion of the skin by microorganism. When it is breached the wound gets contaminated by normal flora and body fluids .Venous ulcer are colonized by aerobic and anaerobic flora.The effect of bacterial burden is called as bioburden. This initiates proinflammatory cytokines like interleukin -1,tumour necrosis factor-α,MMP-2 ,MMP-8.[19]

All chronic ulcers contain some degree of bioburden. Some wounds are infected.Recognizing the range of bioburden in the wounds provides a framework to assess the significance and identify the treatment modalities. The continuum of bioburden are[19] [20]

1. Contamination-Presence of nonreplicating microorganisms on the wound surface.Microorganisms may be endogenous or exogenous.Most common contaminants are *Staphylococcus aureus, Cornyebacterium spp other than Cornyebacterium diptheriae, Coagulase–Negative Staphylococcus, Escherichia coli, Klebsiella spp, Proteus spp* and Anaerobic organisms such as *Prevotella, Bacteroides spp, Peptostreptococcus spp*.

2. Colonization-Microorganisms adhere to the wound's Surface and replicate.Colonization does not impair wound healing.Inappropriate use of

antibiotics during this phase contributes to thegrowth of antibiotic resistant organisms.

3. Critical colonization-The skin cell proliferation and tissue repair areaffected by the bacterial level in the wound. This leads to nonhealing wounds.[21]

4. Biofilm –It is a complex structure of microorganism embedded in an extracellular matrix of polysaccharide.70% of chronic wounds form biofilm. *Staphylococcus aureus and Pseudomonas aeruginosa*[22] are the commonest organism causing chronic biofilm.

5. Infection-It occurs when microorganisms on the wound surface penetrate into wound tissue . A local or systemic response indicates an infection.

The bacteria on the ulcers cause deterioration of wound healing. The bacterial population present within venous leg ulcer (VLU) with severe infection are *methicillin-resistant Staphylococcus aureus* (MRSA), *Streptococcus pyogenes, vancomycin resistant Enterococcus,* Gram-negative bacteria including, *Pseudomonas species, Escherichia coli, Acinetobacter species*, *Klebsiella pneumoniae* and other organisms. The number of species present in the ulcer, rather than one particular bacterial species, correlates positively with impaired healing[23]

Diagnosis of wound infection is based primarily on clinical assessment and microbiological diagnosis. Wound culture is performed primarily to identify the

specific aerobic and anaerobic organisms present and their antimicrobial susceptibility patterns.

Criteria for colonization and infection is [21] [19]

Critical colonization(NERDS)	Deep tissue infection(STONEES)
N-Nonhealing of the wound	S-Size of ulcer larger
E-Exudative wound	T-temperature increased
R-Red and bleeding	O-Osteomyelitis (Probes to or exposed bone
D-debris	N-New area of breakdown
S-Smell from the wound	E-Erythema/Edema
	E-Exudate
	S-Smell

Quantification of the organisms wound is done to assess the levels of bioburden. A quantitative tissue biopsy is the gold standard. Bacterial count of $\geq 10^6$Colony forming units (CFU) per gram tissue is taken as gold standard for infection in venous leg ulcers.[17]

Different types of wound culture done are

CULTURE OF TISSUE

A deep-tissue or punch biopsy for a quantitative culture (which determines the colony counts per gram of tissue) is the gold standard for identifying wound

bioburden and diagnosing clinical infection .After initial debridement and cleaning of superficial debris with normal saline solution,a deep-tissue biopsy is taken.This is the most useful way to detect invasive organisms.[24]Semiquantitative analysis can also be done.

CULTURE OF SWAB

The commonest technique used for evaluating wound infection is the surface swab culture. Levine technique is the best technique for swab culture [25].Here the wound is cleansed of surface exudates with moist saline gauze.A sterile culture swab is then pressed and rotated over an area of $1cm^2$ of the wound .This is done to bring the wound fluid (discharge) and bacteria to the surface.It can be analysed qualitatively and Semiquantitatively. Alginate tipped swab is used for quantitative analysis.

Another variant of this technique is the Z –stroke technique.The surface is swabbed in a Z shape[25] .This is less precise than the Levine's technique.

Surface swabs do not adequately reflect the invasive bacterial organisms They lack the high sensitivity that wound biopsy achieves.

There is lot of debate and controversy regarding the type of sample, sampling techniques and relevance of wound cleaning before sampling[26].Many research works have been done comparing the swab to tissue culture in chronic wounds.

NEEDLE ASPIRATES[26]

When large volume of pus is present, aspiration can be done along the wound margin ,after cleaning the wound.This is a useful method.

TRANSPORT OF SPECIMEN[26]

Prompt delivery of the specimen to the laboratory is important for the viability of the specimen. Specimen for anaerobic study should be transported in prereduced nonnutritive transport medium Tissue samples and aspirates are considered to be preferable to swabs as they retain the environment for the microbial viability.

DIRECT MICROSCOPY[26]

Gram staining of known volume of tissue biopsy specimen homogenate is used to estimate the microbial load of an ulcer. It is a rapid method to facilitate identification of Causative organisms in a clean wound .Its value in chronic wound is interpreted with the presence of leucocytes.

ANALYSIS OF WOUND SPECIMEN

Clinical information regarding the nature of specimen, position of the venous ulcer wound, clinical signs of infection,associated comorbid condition ,malodour and antimicrobial therapy will help the microbiologist in the processing and analyzing the specimen.

ANAEROBIC CULTURE

Anaerobes are commonly isolated from venous ulcers. Quantitative culture for anaerobic organisms is problematic and less meaningful. They are secondary invaders and tend to live in synergy with other organisms in culture.[27][28]

ANTIBIOTIC RESISTANCE AMONG BACTERIA IN VENOUS ULCERS

Bacterial resistance is becoming increasingly common in VLU infection. Risk factors for development resistance pattern include previous antibiotic therapy and its duration, increased frequency and duration of hospitalization.[29]The patients with MRSA can serve as a reservoir for cross contamination through aerosol spread and through health care personnel who are involved in changing the dressing[30]There is increase in true community-acquired MRSA (CA-MRSA) in the long standing venous ulcers. *Pseudomonas aeuginosa* have intrinisic and acquired antibiotic resistance making it difficult to treat.

The Quorum sensing molecules are associated with biofilm formation and the regulation of virulence factors[31].*Staphylococcus aureus and Pseudomonas aeruginosa* are the commonest organisms to form a biofilm. The moist surface of ulcers is favourable for the formation of biofilms.Biofilms confer greater resistance to amtimicrobials and make them less susceptibile to host response.

ANTIMICROBIAL TREATMENT OF VENOUS ULCERS

Systemic antibiotics are indicated only when there is a clinical signs of infection .No routine use of antibiotics are advocated. The guidelines do not advocate the use of topical antibiotics .The topical use of antibiotics have led to resistant organisms.[30] There are also concerns regarding the toxicity and sensitization of the tissue with the use of topical antibiotics. The wounds that are heavily colonized and present with local signs of infection may be treated with topical antibiotics.[32]Short course of metronidazole gel can be given for the odoriferous ulcer.[30]

TREATMENT

A. Compression therapy

The compression therapy has been a fundamental treatment component for Venous leg ulcers.[1]

Compression therapy (CT) is defined as the direct application of external pressure to the limb with the idea of improving the signs and symptoms of chronic venous insufficiency.Methods of compression therapy are [33]

1. Compressive bandages – multilayer bandaging-effective treatment available
2. Compression stockings
3. Intermittent pneumatic compression (IPC)
4. Unna ’s boots and velcro– band devices(legging orthosis)

B. Wound care[17]

a. Regular cleaning and debridement

b. Ulcer dressings: It is of three categories: Passive (nonocclusive), Interactive (semiocclusive and occlusive types), Active (biological types)

The passive dressings (nonocclusive dressings) protect the wound from trauma and potential infection. An example of nonocclusive dressing is dry gauzewith pad and bandage.

The interactive types of dressings maintain a moist warm wound environmentand help to control the amount and composition of wound exudate.

They may be semiocclusive or occlusive type.

The common types of semi occlusive/occlusive dressings include hydrocolloids, hydrogels, films, foam, and calcium alginates. The active or biologicdressings may be living human dermal equivalent (LHDE), platelet products – autologous or recombinant and growth factors (epidermal growth factor; GMCSF, etc.).

There is no ideal dressing material for venous ulcers. The saline wet to dry gauze dressing is a simple and popular form

C. Management of pain

Venous ulcers are generally considered to be painless unless complicated by infection. Pain is managed by compression treatment and antimicrobial therapy.

D. Skin grafting for venous ulcers[17].

Split-thickness skin grafting is done for large ulcers requiring extendedtime to heal.

E. MEDICATIONS

Pentoxifilline ,Vitamin A and E,Calcium channel blockers,Aspirin and corticosteroids whenever necessary

F. SURGERY

Surgical correction of the underlying cause of chronic venous insufficiency is considered whenever possible.

G. PATIENT EDUCATION[34]

- Elevation of foot for at least 30 minutes a day.
- Avoidance of smoking
- Reduction of Overweight

MATERIALS AND METHODS

This study on secondary bacterial infections in venous leg ulcers patients and their antimicrobial susceptibility pattern was carried out in the Institute of Microbiology, Madras Medical College ,Chennai

Study design & period

Cross sectional study. From October 2014 to August 2015

Study population

A total number of 100 patients attending the department of department of Vascular surgery, General surgery and Plastic Surgery, Rajiv Gandhi Government General Hospital, Chennai were included for the study.

Ethical clearance

Prior approval was obtained from the Institutional Ethics Committee. Informed consent was obtained from the in-patients and out patients who satisfied the inclusion criteria.

Inclusion criteria

- Patients older than 18 years.
- IP/OP Patients with Venous leg ulcers with one or more of the following clinical signs of infections

  Fever

Increased pain

Discharge

Malodour

Increased oedema

Exclusion criteria:

- Patients with arterial ulcers,Filarial ulcers
- Patients with neurotrophic ulcers- Diabetic ulcer, Leprotic ulcer
- Patients with venous leg ulcers having no clinical signs of infection.

COLLECTION OF DATA

Data were collected from patients who satisfied the inclusion criteria.Demographic details like name, age, sex, address, date of admission, clinical data like presenting complaints, personal history, past medical history, immunocompromised status, physical examination findings and details of clinical diagnosis and investigations were collected.

METHODLOGY FOLLOWED IN THE STUDY

As the Quantitative culture of the tissue is the Gold standard,This procedure was followed in this study.

SAMPLE COLLECTION AND TRANSPORT

Samples collected

Tissue: The ulcer was cleaned with normal saline.Under aseptic precautions two tissue bits were collected from the ulcer bed with a sterile scalpel.One tissue bit was transferred into a sterile universal container and another bit into Robertson's cooked meat broth and transported to the laboratory.

PROCESSING OF SPECIMEN

TISSUE[35]

The tissue was weighed in a preweighed sterile petridish on an analytical balance. Tissue was homogenized with a sterile scalpel in the petridish and transferred to a sterile test tube containing 5 ml of 0.85% normal saline and vortex mixed.

DIRECT GRAM STAIN[26]

0.2 ml of tissue homogenate was applied on a clean glass slide and was spread as a thin smear. It was allowed to air dry for 15 minutes and heat fixed. Gram staining was done andthe smear was examined with 100x oil immersion objective.

The presence of a single organism per field was regarded as equivalent of the 10^5 bacteria per gram of tissue. Gram stain morphology and presence of any pus cells were documented.

Processing of tissue for quantitative culture[35]

The tissue homogenised was transferred into a sterile test tube containing 5 ml of 0.85%normal saline and was vortex mixed .It was plated onto MacConkey Agar and serially diluted 10 folds before plating onto Blood agar. The plates were incubated at 37°C aerobically.

The number of Colony Forming Units (CFU) per gram of tissue was calculated by applying the following formula:

Number of CFUs counted x Reciprocal of volume of homogenate inoculated (10^{-1} or 10^{-2}) x 2 (volume of diluents used for tissue homogenization) divided by the weight of tissue in grams.

Interpretation: ---------- CFU/ gram tissue

Anaerobic culture

For anaerobic culture, the tissuewas inoculated directly into Robertson cooked meat broth(RCM) and transported to the laboratory.

Processing of specimen: [36]

Tissue bits were inoculated onto freshly prepared e anaerobic blood agar plates(Appendix-II) and placed in anaerobic jar with media facing upwards. AnaeroGas (HiMedia)was placed inside the McIntosh Fildes anaerobic jar and the lid of the jar closed immediately .Lid was sealed with petroleum jelly and kept for

incubation for 48 hours at 37ºC.A blood agar plate inoculated with ATCC *Pseudomonas aeruginosa-27853* was placed in the jar which served as a control to check anaerobic process.

After 48-72 hours, all primary plates were examined .Identification of anaerobic bacteria was one by Colony morphology, Gram's staining and by using antibiotic identification disks.The culture was subcultured onto anaerobic blood agar plates with following antibiotic identification disks

- Kanamycin-1 mg
- Colistin-10 µg
- Vancomycin-5µg
- Nitrate disk for Gram negative organism and Nitrate disk and Sodium Polyanetholsulfonate (SPS) disk were added for Gram positive organismsand incubated anaerobically in McIntosh Fildes jar for 48-72 hours.

Colonies were also subcultured onto chocolate agar plate and incubated in 5% carbon dioxide in a candle jar for aerotolerance at 37ºC.Since no growth was observed after 48 hours,the isolate was considered as obligate anaerobe.

IDENTIFICATIONOF ISOLATES

All the bacterial isolates obtained from the samples were identified by standard bacteriological techniques

Beta haemolytic colonies and golden yellow pigment on blood agar, Gram positive cocci in clusters on Gram stain, positive catalase test, positive slide coagulase test, positive tube coagulase test, positive urease test, fermentation of mannitol, positive Methyl Red (MR) test, positive Voges Proskauer (VP) test, and production of phosphatase were identified as *Staphylococcus aureus*[38]

Staphylococcus epidermidis was identified by, white opaque colonies on blood agar, Gram positive cocci in clusters on Gram stain, positive catalase test, negative slide coagulase test, negative tube coagulase test, production of phosphatase, sensitive to Novobiocin, resistant to Polymyxin B and negative mannitol fermentation[38]

Micrococci is identified by ,lactose fermenting colonies on MacConkey agar, Gram positive cocci in tetrads, positive catalase test, modified oxidase test positive.[38]

Streptococcus pyogenes was identified by, beta hemolysis on blood agar, Gram positive cocci in pairs and short chains ,Gram stain, negative catalase test, sensitivity to bacitracin 0 .04 units ,negative bile esculin test.[39]

Enterococcus faecalis was identified by, non-haemolytic tiny colonies on blood agar, Gram positive cocci in pairs and short chains on Gram stain, negative catalase test, positive bile esculin test, positive arginine dihydrolase test, fermentation of mannitol, non-fermentation of arabinose, heat tolerance (surviving at 60^{o}C for 30 min)[39].

Diphtheroids were identified by white opaque colonies on 5% Blood agar,Gram positive bacilli on Gram stain,non motile detected by hanging drop method,positive catalase test,negative oxidase test, and with Urease test, Nitrate reduction test, Fermentation of glucose, Esculin hydrolysis, Arginine dihydrolase test, VP test and fermentation of sugars.[43]

Escherichia coli was identified by, lactose fermenting colonies on MacConkey agar, Gram negative bacilli on Gram stain, motile bacilli detected by hanging drop method, positive catalase test, negative oxidase test, positive nitrate reduction test, positive indole test, positive MR test, negative VP test, negative citrate utilization test, acid butt and acid slant with gas on TSI, negative urease test and fermentation of sugars with acid and gas.[40]

Proteus vulgaris was identified by ,non lactose fermenting colonies on MacConkey agar, Grey white colonies with swarming in Blood agar,Gram negative bacilli on Gram stain,motile bacilli detected by hanging drop method, positive catalase test, negative oxidase test, positive nitrate reduction test, positive indole test, positive MR test, negative VP test, positive citrate test, acid butt and alkaline slant with H_2S production on TSI,Positive urease test ,positive phenylalanine deaminase test and fermentation of sugars.[40]

Proteus mirabilis was identified by ,non lactose fermenting colonies on MacConkey agar, Grey white colonies with swarming in Blood agar,Gram negative bacilli on Gram stain,motile bacilli detected by hanging drop method,Positive

catalase test, negative oxidase test, positive nitrate reduction test, negative indole test, positive MR test, negative VP test, positive citrate test, acid butt and alkaline slant with H_2S production, on TSI,Positive urease test ,positive phenylalanine deaminase test and fermentation of sugars,positiveOrnithine decarboxylation[40]

Klebsiella oxytoca was identified by lactose fermenting mucoid colonies on MacConkey agar, short Gram negative bacilli on Gram stain, non-motile bacilli detected by hanging drop method, positive catalase test, negative oxidase test, positive nitrate reduction test, positive indole test, negative MR test, positive VP test, positive citrate utilization test, acid butt and acid slant with gas on TSI, positive urease test and fermentation of sugars with acid and gas.[40]

Klebsiella pneumonia was identified by lactose fermenting mucoid colonies on MacConkey agar, short Gram negative bacilli on Gram stain, non-motile bacilli detected by hanging drop method, positive catalase test, negative oxidase test, positive nitrate reduction test, negative indole test, negative MR test, positive VP test, positive citrate utilization test, acid butt and acid slant with gas on TSI, positive urease test and fermentation of sugars with acid and gas[40].

Pseudomonas aeruginosa was identified by, non-lactose fermenting colonies on MacConkey agar, production of bluish green pigment on nutrient agar, slender Gram negative bacilli on Gram stain, motile bacilli detected by hanging drop method, positive catalase test, positive oxidase test, oxidative reaction in Hugh & Leifson Oxidative and fermentative medium, positive nitrate reduction test, negative

MR test, negative VP test, alkaline butt and alkaline slant with gas on TSI, positive arginine dihydrolase test and negative lysine decarboxylation[41]

Acinetobacter baumannii was identified by, non-lactose fermenting colonies on MacConkey agar, Gram negative coccobacilli on Gram stain, nonmotile bacilli detected by hanging drop method, positive catalase test,negative oxidase test, oxidative reaction in Hugh & Leifson O/F medium,10% OF lactose positive,negative nitrate reduction test, negative MR test, negative VP test, alkaline butt and alkaline slant on TSI, Growth at 42°C.[42]

IDENTIFICATION OF ANAEROBES[36]

Interpretation of antibiotic identification disk method for anaerobic identification

Organism	Gram Stain	Vancomycin 5µg	Kanamycin 1 MG	Colistin 10	Nitrate	SPS	Catalase
Peptostreptococcus anaerobicus	Gram positive cocci Or Coccobacilli	S	R	R	-	S	-
Bactroides fragilis	Gram negative bacilli	R	R	R	+	-	-

ANTI MICROBIAL SUSCEPTIBILITY TESTING[44][45]

All aerobic Bacterial isolates were tested for antimicrobial susceptibility pattern using Kirby –Bauer Disc diffusion Method.

ANTIMICROBIAL SUSCEPTIBILITY PATTERN TESTING BY KIRBY-BAUER DISC DIFFUSION METHOD

Inoculum Preparation and procedure

3-5 similar colonies from 24 hour culture was transferred to a sterile testtube containing 3 ml of peptone water with the help of sterile bacteriological loop.

The same was emulsified and turbidity matched with 0.5 McFarlands standards.

1. By using Sterile cotton swab, The suspension was evenly streaked over cation adjusted Mueller Hilton agar in three directions approximately at 60° to evenly distribute the inoculum.

2. Antibiotic disks (HiMedia) were placed on the agar plate after allowing the plates to dry for 3-5 minutes.(Appendix) with a sterile forceps.

The petridishes were incubated overnight at37℃ aerobically for 24 hours.The diameter of Zone of inhibition were read with the ruled template.

Interpretation was done according to the CLSI guidelines.(Appendix-III)

For diphtheroids, A panel of three drugs were tested and interpreted according to the British society of antibiotic chemotherapy (BSAC) (Appendix –III)

Quality control tests were done every week with following ATCC strains to test the efficacy of media and drugs.

ATCC control strains:

- *Staphylococcus aureus*–ATCC 25923
- *Escherichia coli*-ATCC 25922
- *Pseudomonas aeruginosa*-ATCC 27853
- *Klebsiella pneumoniae*(ESBL)-ATCC 700603

Identification of Methicillin resistant staphylococcus aureus[45]

Screening test

3-5 colonies from overnight culture was transferred to 2 ml peptone water and emulsified .The turbidity was matched with 0.5 McFarlands standard.

Lawn culture was made with the same on Muller Hilton agar plate and Incubated overnight at 33-35°C.Cefoxitin 30µg disk was placed on the agar plates. The Zone of Inhibition was interpreted according to CLSI guidelines.

A similar lawn culture of ATCC *Staphylococcus aureus* 25923 was put up as Quality control strain.

Interpretation of Zone of Inhibition

Organism	**Methicillin Sensitive**	**Methicillin Resistant**
Staphylococcus aureus and *Staphylococcus lugdunensis*	≥22 mm	≤21mm
Coagulase negative staphylococcus	≥25 mm	≤24 mm

DETERMINATION OF MINIMUM INHIBITORY CONCENTRATION (MIC) BY MACROBROTH DILUTION METHOD FOR VANCOMYCIN RESISTANCE[46][47]

Requirements

1. Culture media: Cation adjusted Mueller Hilton (MH) broth (pH7.2-7.4)

Preparation of antibiotic stock solution:

Vancomycin used for preparing the stock solution was obtained from HiMedia

Weight of antibiotic for preparation of stock solution was calculated by the formula

$$W = \frac{1000}{P} \times V \times C$$

P = Potency of the antibiotic in relation to the base (For Vancomycin, P=950/1000)

V= Volume of the stock solution to be prepared

C=Final concentration of antibiotic solution (1024 µg/ml)

W= weight of the antibiotic to be dissolved in the volume V

10.8 mgs of the drug is added to 10 ml of the distilled water for the final concentration 1024 µg/ml.

PREPARATIONOFANTIBIOTIC DILUTION

1. Two rows of 14 sterile test tubes were arranged in a rack.First row for Test organism and second for ATCC control.
2. 1 ml of MH broth was transferred to all the tubes in the rack using micropipette.
3. 1 ml of Stock solution was transferred to the first test tube in each row and mixed well.
4. 1 ml from the first test tube was transferred to the second tube and serially diluted till 14^{th} tube.
5. One tube containing only antibiotic solution was kept for drug control.
6. Inoculum Preparation: To 9.9 ml of MH broth 0.1 ml of 0.5 McFarland turbidity organism was added and mixed well. From the above prepared inoculum 1 ml was transferred to each tube kept for test organism.One tube containing only the test inoculum was kept as controlSame procedure was repeated for ATCC control strain
7. The test tubes were incubated at 37°C overnight.

INTERPRETATION[45]:

MIC of ATCC *Staphylococcus aureus* 25923 and the test organism was observed

The lowest concentration of the antibiotic which showed clearing was considered as the MIC for the ATCC strain and for the test organism was interpreted as follows according to CLSI guidelines

MIC of Vancomycin

≤ 2 µg/ml- Susceptible

4-8 µg/ml-Intermediate

≥ 16 µg/ml-Resistant

ANTIMICROBIAL SENSITIVITY TO CLINDAMYCIN[45]

INDUCIBLE D TEST

The antimicrobial sensitivity to Clindamycin for Gram positive cocci was done as follows

A lawn culture of the isolate was done after matching the inoculum with 0.5 McFarlands Standard. Erythromycin 15µg disk and Clindamycin 2µg disk were placed 15 mm apart and incubated at 37ºC for 18 hours.

Interpretation

Hazy growth within the zone of inhibition-Clindamycin resistant

Flattening of zone of inhibition adjacent to the erythromycin disk was considered as inducible Clindamycin resistance

The isolates were reported as sensitive to Clindamycin only when there was no inducible resistance to Clindamycin

DETECTION OF BIOFILM FORMATION BY METHICILLIN REISTANT STAPHYLOCOCCUS AUREUS BY TISSUE MICROTITRE PLATE[48][49][50]

1. 1-2 colonies from fresh agar plate of test organism were inoculated in 3 ml of Trypticase soy broth (TSB) with 1% glucose and incubated for 24 hours at 37°C .
2. The inoculum for the biofilm detection was diluted with fresh Trypticase soy broth so that final concentration corresponded to 1 in 100 dilution
3. 0.2 ml of the diluted inoculum was transferred to the individual wells of sterile Polystyrene ,96 well-Flat bottom tissue culture plates (TARSON)
4. Controls were set up in individual well- Blank well, crystal violet, sterile Trypticase soy broth, fixative.
5. ATCC *Pseudomonas aeruginosa*-ATCC 27853 and ATCC *Staphylococcus aureus*-ATCC 25923 were set up as the Positive control and negative control for biofilm producer respectively
6. The test was done in triplicates and the plates were incubated at 37°C for 24 hours at 37°C.
7. After incubation, the contents of the well were removed by gentle tapping.

8. The wells were washed with 0.2 ml of Phosphate buffer solution (PBS) for four times. This was done to remove the planktonic bacteria.

9. The biofilm thus formed was fixed to the plate with 2 % sodium acetate and stained with 250 µl of 0.1 % crystal violet and incubated at room temperature for 20 minutes.

10. Excess stain was rinsed off by washing with 250 µl of deionised water for four times.

11. 200 µl of 95%ethanol was added to solubilize the Crystal violet and to extract the violet colour to quantify it.

12. Optical density of adherent bacteria were determined with a Micro ELISA auto reader at wavelength of 570 nm (OD_{570nm}).

13. The OD valves were calculated.

    ODC value was calculated using the formula

    ODC (Optical density cut off value) = Average OD of Negative control + 3 x standard deviation of Negative control

14. Interpretation

    Strong ->4 times ODC

    Moderate - 2 times the ODC- 4 Times ODC

    Weak - $\leq$ 2 x ODC

Determination of Extended spectrum beta lactamase (ESBL) production

Screening test [45]

All Gram negative isolates were screened with Two disk Cefotaxime 30µg and Ceftazidime 30µg and considered to be ESBL producers if Zone of inhibition for

Cefotaxime 30µg-≤ 27 mm

Ceftazidime 30µg - ≤ 22 mm

These isolates were subjected to phenotypic confirmatory test.

Lawn culture of the isolates were made on Mueller Hilton agar plate. Ceftazidime 30µg, Ceftazidime- Clavulanate 30µg/10µg disks and Cefotaxime30µg, Cefotaxime-Clavulanate 30µg/10µg disks were placed and incubated at 37ºC for 18 hours.

INTERPRETATION

An increase in Zone of inhibition by ≥ 5 mm diameter for either antimicrobial agent tested in combination with β Lactamase inhibitor was confirmed as ESBL producer.

DETERMINTAION OF AmpC production[37]

SCREENING TEST

All Gram negative isolates were screened with cefoxitin30µg disk for AmpC production.

Lawn culture of the isolates were made on Mueller Hilton agar plate Cefoxitin30µg disk were placed and incubated at 37ºC for 18 hours.

INTERPRETATION

Zone of inhibition by ≤18 mm diameter for cefoxitin was taken as Amp C production .

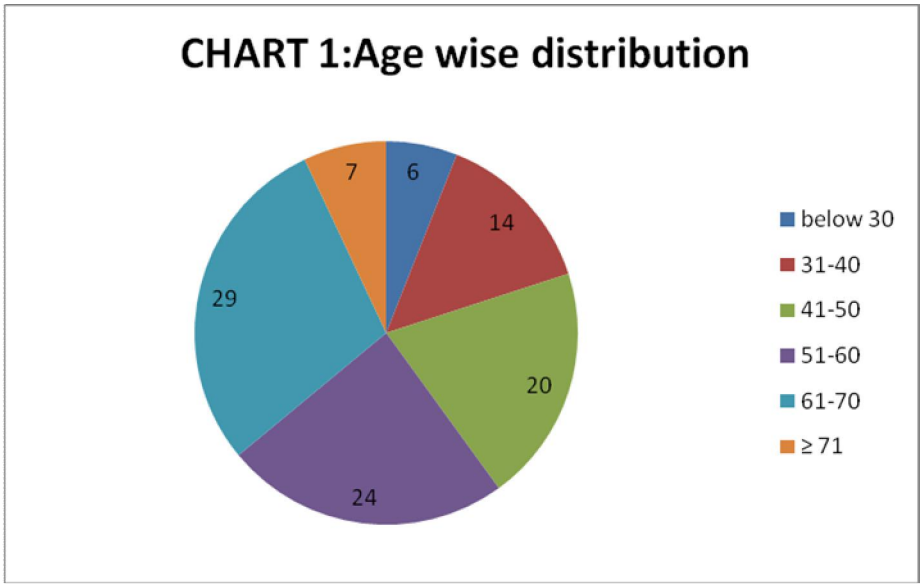
RESULTS

This study was done on 100 patients with venous leg ulcers and results were analysed statistically using SPSS version 2.1.

TABLE 1: AGE WISE DISTRIBUTION OF CASES (n=100)

Years	No of cases	Males	Females	Percentage %
Below 30	6	6	-	6
31-40	14	12	2	14
41-50	20	17	3	20
51-60	24	20	4	24
61-70	29	22	7	29
≥ 71	7	7	-	7
TOTAL	100	84	16	

Out of 100 cases, twenty nine cases were found in the age group of 61-70 years, followed by 51-60 years age group.



Out of 100 patients,Eighty four were Males and sixteen were females.

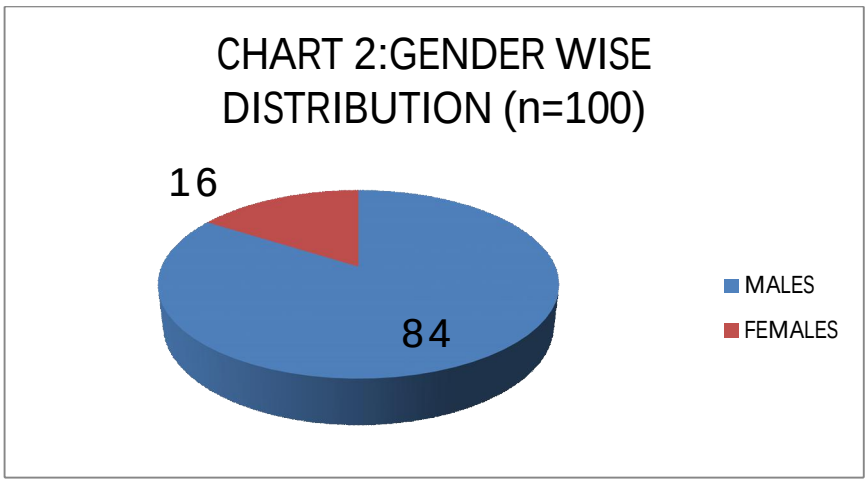


TABLE 2: The anatomical distribution of the venous ulcers (n=100)

Anatomical Location	No of Ulcers	Percentage
Above Medial Malleolus	81	81
Above lateral Malleolus	17	17
Gaiter region	2	2
Total	100	

Most common position of the Ulcers was above the Medial malleolus.This corresponds to the anatomy and pathology of the Venous ulcers

TABLE 3: Comorbid conditions in the study population (n=100)

Diseases	Number	Percentage
Diabetes mellitus	18	18
Hypertension	4	4
Diabetes/Hypertension	4	4
Obesity	12	12
Co morbid Cardiac diseases	4	4
Chronic renal failure	1	1
Herniorhaphy	3	3
No associated comorbid conditions	54	54
Total	100	

Out of 100 patients, twelve patients were obese (above 90 kgs).

TABLE 4 : Occupation of the Patients (n=100)

Type of Occupation	No of patients	Percentage
Security workers	21	21
Daily wagers	17	17
Farmers	10	10
Cooks in hotels	9	9
Tailors	9	9
Vendors	8	8
Supervisors	4	4
Others	22	22
Total	100	100

Among the occupation of the study population, 21 were security workers.17 were daily wagers

TABLE 5: Personal habits of the patients (n=100)

Habits	No of cases	Percentage
Alcoholics	21	21
Smoker	9	9
Alcoholic/smoker	25	25
No habits	45	45
Total	100	

Out of 100 patients 25 were both alcoholics and smokers.

TABLE 6: Association With Venous Pathology(n=100)

Venous pathology	No of cases	Percentage	P value
DVT	10	10	0.001*
Operated either for Varicose veins or SSG done	16	16	0.001*
Visible Varicose Veins	66	66	0.001*
IVC thrombosis operated	1	1	
No pathology	7	7	
Total	100	100	

Ten patients were treated for Deep Vein thrombosis (DVT).Sixteen patients were either operated for varicose veins or had split skin grafting done for the ulcer.66 patients had varicose veins.

TABLE 7: Results of doppler study among the study population.(n=100)

Venous pathology	No of cases	Percentage
Great Saphneous Vein Pathology	22	22
Short saphaneous Vein Pathology	35	35
Perforator Incompetence (Above ankle ,Below Knee, Mid calf, Above Knee)	43	43
Total	100	100

Perforator Incompetence was found in 43 patients.

TABLE 8: Clinical Signs And Symptoms Of Study Population (n=100)

Complaints	No of cases	Percentage
Increased Pain	46	46
Swelling	5	5
Discharge	15	15
Discharge with Malodour	5	5
Pain and Discharge	26	26
Pain and Swelling and Fever	3	3
Total	100	100

The clinical spectrum of the patients were Increased pain,followed by pain and discharge from the wound.Five patients had swelling and three had Fever.

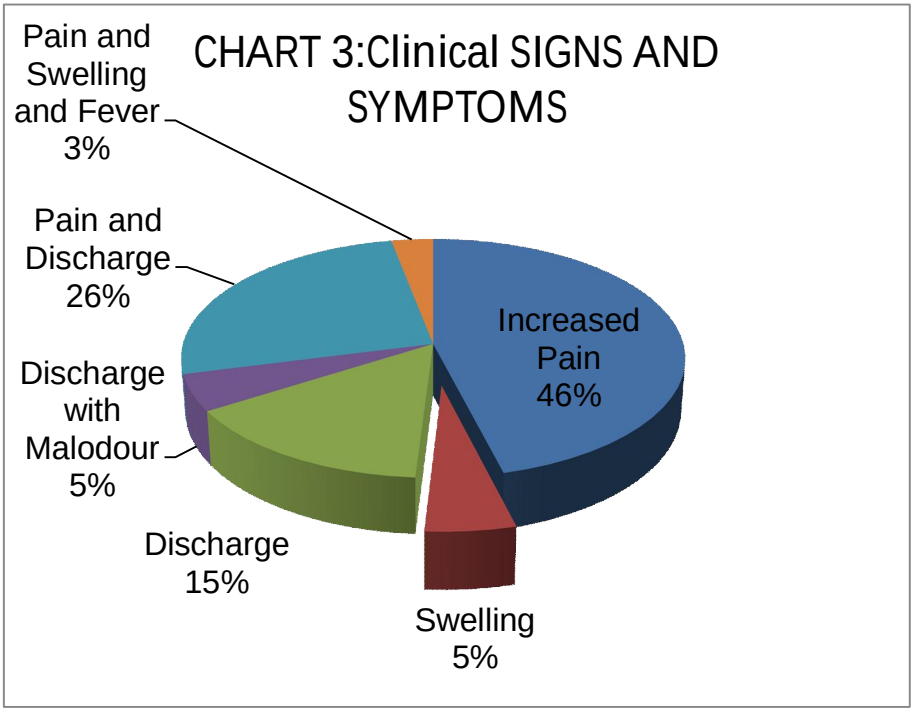


TABLE 9: Correlation of Gram Stain to Culture Positivity

Direct Gram stain was used as screening test for quantitative culture. (n=100)

	Culture Positive	**Culture Negative**	**significance**
Smear positive (Organism ±Pus cells)	53	-	P<0.001
Smear negative (No Organism ± Pus cells)	38	9	

From 100 venous ulcer patients, One hundred and thirty nine bacterial organisms were isolated.

TABLE 10: Microbial Distribution in Ulcers (n=100)

No of Organisms	**No of ulcers**	**No of isolates**	**Percentage**
Monomicrobial	46	46	46
Polymicrobial	45	93	45
No Growth	9		
Total	100	139	

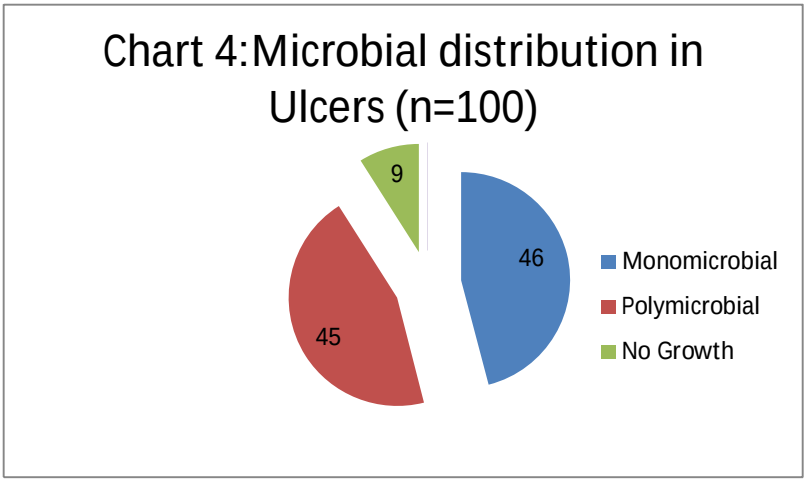
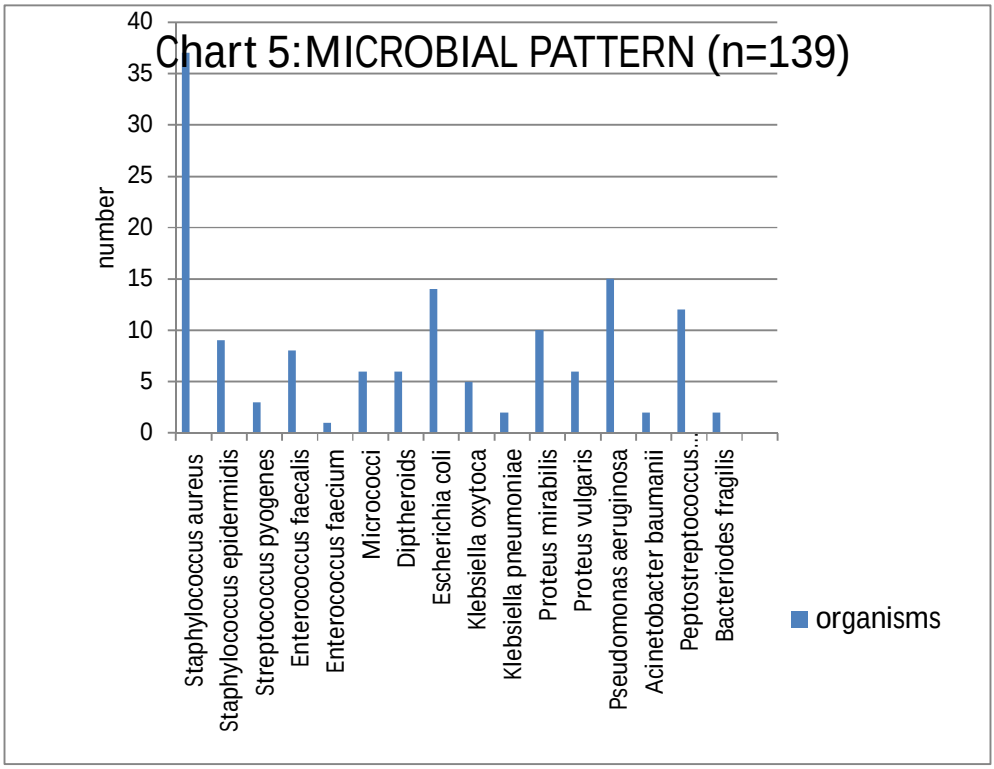


TABLE 11: TOTAL NUMBER OF ISOLATES (n=139)

Name of the Organism	No of isolates	Percentage (%)
Staphylococcus aureus	37	26.6
Staphylococcus epidermidis	9	6.4
Streptococcus pyogenes	3	2.1
Enterococcus faecalis	8	5.7
Enterococcus faecium	1	0.71
Micrococci	6	4.3
Diptheroids	6	4.3
Escherichia coli	14	10.07
Klebsiella oxytoca	5	3.5
Klebsiella pneumoniae	3	2.1
Proteus mirabilis	10	7.1
Proteus vulgaris	6	4.3
Pseudomonas aeruginosa	15	11.1
Acinetobacter baumanii	2	1.4
Peptostreptococcus anaerobicus	12	8.6
Bacteriodes fragilis	2	1.4
Total	139	



Staphylococcus aureus was the commonest organism to be isolated. Out of 139 organisms isolated,37 were *Staphylococcus aureus.*

Out of 139 isolates, Forty two aerobic isolates were found to have colony counts $\geq 10^6$CFU/gram tissue and 14 anaerobes were isolated .

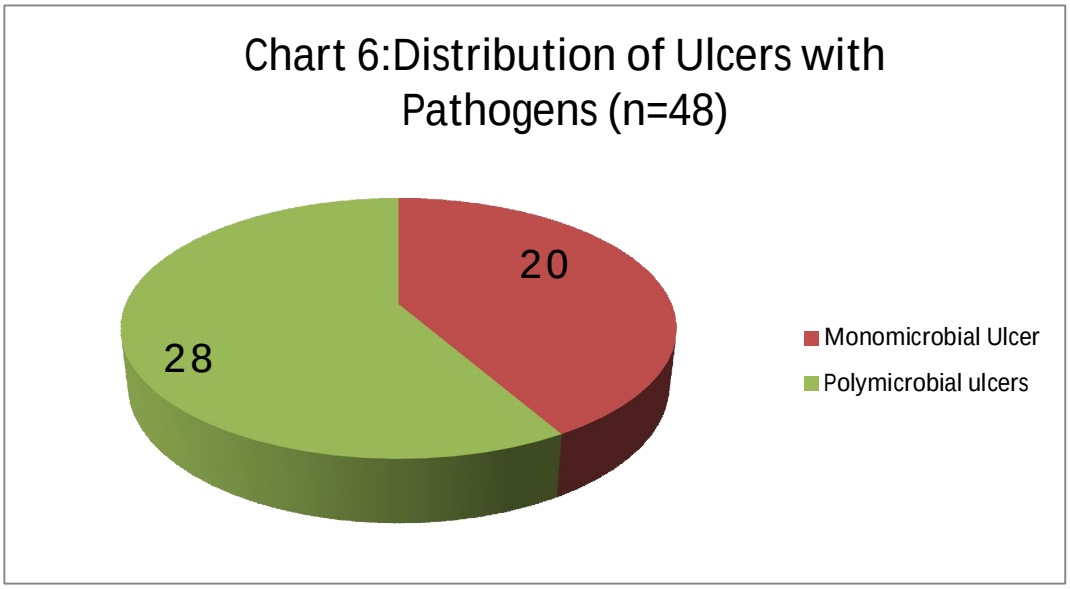
Table 12: Distribution of Infection in the ulcers(n=100)

Type of Ulcer	No of Ulcers	Infected Ulcers	Percentage
Monomicrobial	46	20	20
Polymicrobial	45	28	28
No growth	9		
Total	100	48	

TABLE 13: Distribution of Pathogens (n=56)

Type of ulcer	No of Pathogens		Percentage
	Aerobes	Anaerobes	
Monomicrobial Ulcer	20	-	35.71
Polymicrobial ulcers	22	14	64.29
Total (n=56)	42	14	

Anerobes were isolated from 14 ulcers.



Distribution of Pathogens in the Ulcers (n=56)

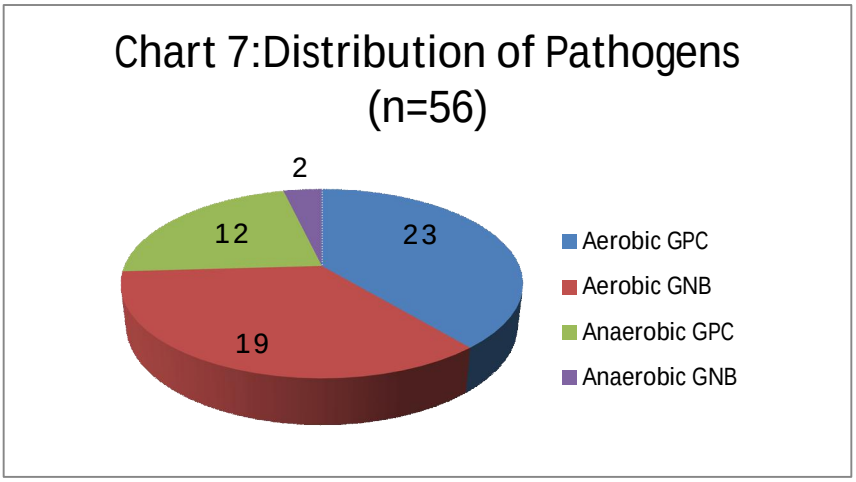


TABLE 14: Distribution of Aerobic Pathogens (≥10^6CFU/gram tissue) among the ulcers (n=42)

Organism	Monomicrobial Ulcers	Polymicrobial Ulcers	Total	%
Staphylococcus aureus	12	5	17	40.47
Staphylococcus epidermidis	1		1	2.3
Streptococcus pyogenes	2	1	3	7.14
Enterococcus faecalis		2	2	4.7
Escherichia coli	2	6	8	19.04
Proteus vulgaris		1	1	2.3
Proteus mirabilis		3	3	7.14
Klebsiella oxytoca	1	1	2	4.7
Pseudomonas aeruginosa	2	3	5	11.9
Total (n=42)	20	22	42	

The pathogenicity was determined by the colony count. *Staphylococcus aureus* was found to be pathogen in 17 ulcers .Out of 17 *Staphylococcus aureus* isolates, 13 were methillicin resistant .ESBL producers were found in seven ulcers.

Streptococcus pyogenes was isolated in three cases. It was found to be single organism infecting the wound in two ulcers. *Streptococcus pyogenes* were taken as pathogen irrespective of colony count.

Chart 8: Distribution of aerobic Pathogens (n=42)

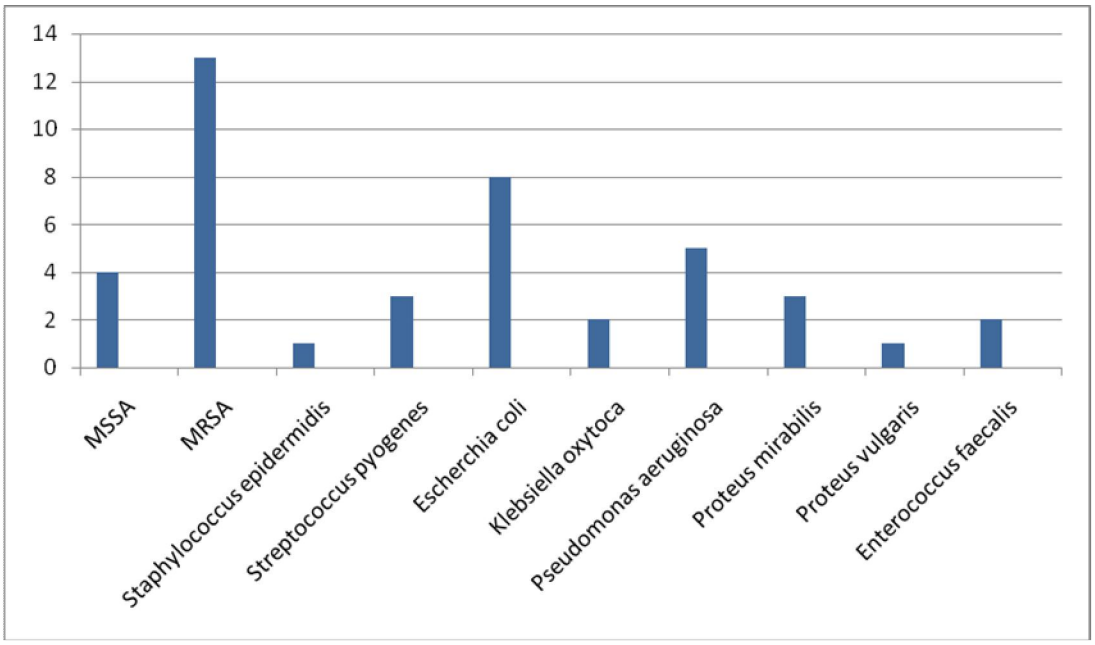
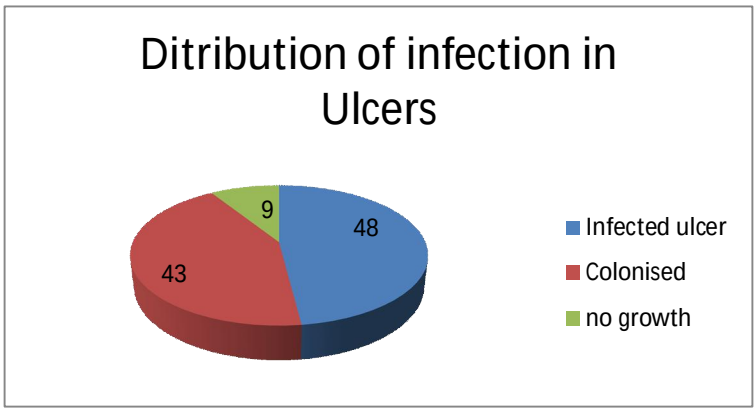


Chart 9: Distribution of infection and colonization in ulcers (n=100)



Out of 100 ulcers, 43 % was found to be colonized ($\leq 10^5$CFU/gram tissue). In 43 ulcers, 83 aerobic isolates were found to be colonizers (59.7%).

Table 15: Distribution of Colonisers (n=83)

Name of Organism	No of isolates	Percentage
Staphylococcus aureus	20	24.8
Staphylococcus epidermidis	8	9.6
Enterococcus faecalis	6	7.2
Enterococcus faecium	1	1.2
Micrococci	6	7.2
Diptheroids	6	7.2
Escherichia coli	6	7.2
Klebsiella oxytoca	3	3.6
Klebsiella pneumoniae	3	3.6
Proteus mirabilis	7	8.4
Proteus vulgaris	5	6.02
Pseudomonas aeruginosa	10	12.4
Acinetobacter baumanii	2	2.4
Total	83	

IDENTIFICATION OF ANAEROBES

Fourteen Anaerobes were isolated from fourteen ulcers.They were isolated along with the aerobic organism in the ulcers. *Peptostreptococcus anaerobicus* was isolated from 12 ulcers and *Bacteroides fragilis* from two ulcers. The Quantification was not done for anaerobes.

TABLE 16: Distribution of the anaerobes (n=14)

Organism	No of cases
Peptostreptococcus anaerobicus	12
Bacteroides fragilis	2
Total	14

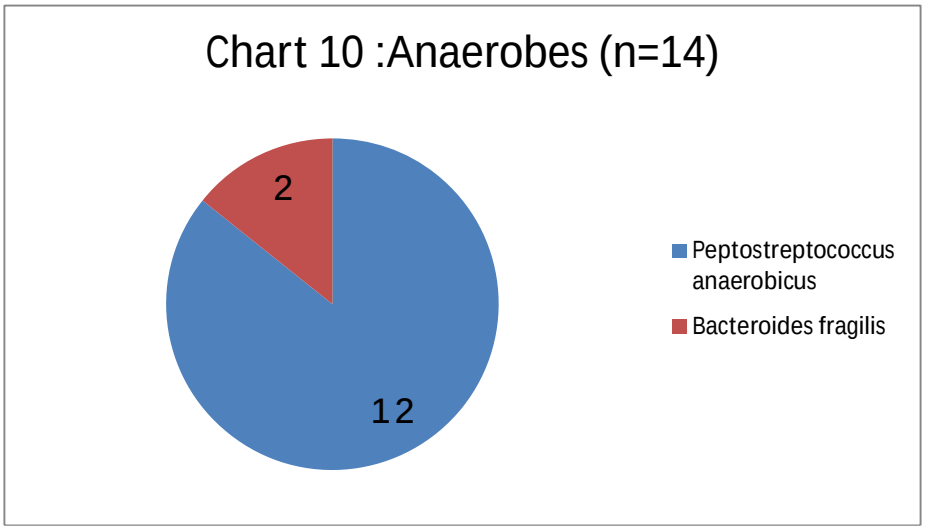


TABLE 17: Duration of the venous ulcers (n=100)

Duration	No of cases	Infected cases(n=48)	Percentage
Below 1 year	4	2	4
1-5 years	35	16	35
6-10 years	36	20	36
11-15 years	18	7	18
≥ 16 years	7	3	7
Total	100	48	100

Out of 100 patients, thirty six patients had ulcer for 6-10 years duration.Seven patients had the ulcer for more than 16 years. Out of 36 ulcers in 6-10 Years of duration, 20 ulcers were found to be infected. The duration of ulcer did not correlate with the infection.

P valve is < 0.038. It is not significant.

ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF THE ISOLATES.

Antimicrobial susceptibility pattern of the isolates were determined by disk diffusion method and MIC determination for Vancomycin and interpreted according to the CLSI guidelines. Antimicrobial susceptibility pattern was not done for Anaerobes.Micrococci were taken as normal skin commensals**.**

ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF THE ISOLATES.

TABLE 18: Gram Positive Organisms(n=64)

ORGANISM	PEN 10µg	ERY 15µg	AK 30µg	CIP 5µg	COT 1.25/23.75 µg	CX 30µg	GM 10µg	TET10 µg	VAN 30µg	CL 2µg*	HLG 120 µg
S.aureus (MSSA) (24)	100% (24)	79.2% (19)	87.5% (21)	62.5% (16)	70.8% (17)	100% (24)	83.3% (20)	91% (22)	NT	100% (24)	NT
S.aureus (MRSA) (13)	0% (13)	30% (4)	76.9 (10)	61.5% (8)	23.8% (3)	0% (13)	69.8% (9)	30% (4)	NT	100% (13)	NT
S.epidermidis (9)	100% (9)	100% (9)	77.7% (7)	66.6% (6)	66.6% (6)	100% (9)	66.6% (6)	88.8% (8)	NT	100% (9)	NT
Streptococcus Pyogenes (3)	100% (3)	100% (3)	NT	100% (3)	NT	NT	NT	100% (3)	100% (3)	100% (3)	NT
Enterococcus faecalis (8)	100% (8)	62.8% (5)	NT	100% (8)	NT	NT	NT	NT	100% (8)	NT	100% (8)
Enterococcus faecium (1)	100% (1)	100% (1)	NT	0% (0)	NT	NT	NT	NT	100% (1)	NT	100% (1)
Diphtheroids** (6)	100% (6)	NT	NT	100% (6)	NT	NT	NT	NT	100% (6)	NT	NT

*Clindamycin sensitivity was reported after testing with Inducible D test according to the CLSI guidelines.

** Sensitivity to Diphtheroids were interpreted according to British Society ofAntimicrobial Chemotherapy guidelines.Micrococci were treated as normal skin commensals.

KEY TO THE TABLE

PEN- Penicillin, ERY- Erythromycin, AK-Amikacin, CIP-Ciprofloxicin, COT-Cotrimoxazole, CX-Cefoxitin,GM-Gentamicin,TET-Tetracycline,VAN-Vancomycin,CL-Clindamycin,HLG- High level gentamicin

ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF THE ISOLATES.
TABLE 19: Gram Negative Bacilli(n=55)

Organism	AK 30µg	CIP 5µg	COT 1.25/23.75 µg	PT 100/10 µg	CAZ 30 µg	CTX 30 µg	GM 10µg	TET 10µg	IMP 10 µg	CX 30 µg
E.Coli (14)	92.8 (13)	85.75% (12)	57.14% (8)	100% (14)	57.14% (8)	57.14% (8)	85.75% (12)	85.75% (12)	100% (14)	100% (14)
K.oxytoca (5)	60% (3)	60% (3)	60% (3)	80% (4)	80% (4)	80% (4)	60% (3)	60% (3)	100% (5)	100% (5)
K.pneumoniae (3)	33.3% (1)	66.6% (2)	100% (3)	100% (3)	100% (3)	100% (3)	33.3% (1)	100% (3)	100% (3)	100% (3)
Proteus vulgaris (6)	100% (6)	66.6% (4)	83.3% (5)	100% (6)	100% (6)	100% (6)	83.3% (5)	100% (6)	100% (6)	100% (6)
Proteus mirabilis (10)	90% (9)	80% (8)	70% (7)	100% (10)	100% (10)	100% (10)	80% (8)	70% (7)	100% (10)	100% (10)
Pseudomonas aeruginosa (15)	73.3% (11)	60% (9)	NT	100% (15)	100% (15)	NT	66.6% (10)	NT	100% (15)	100% (15)
Acinetobacter baumanii (2)	100% (2)	100% (2)	100% (2)	100% (2)	100% (2)	100% (2)	100% (2)	100% (2)	100% (2)	100% (2)

KEY TO THE TABLE
AK-Amikacin,CIP-Ciprofloxicin,COT-Cotrimoxazole, CX-Cefoxitin,GM-Gentamicin,TET-Tetracycline,PT-Piperacillin-Tazobactam,CAZ-Ceftazidime,CTX-Cefotaxime, IMP- Imipenam

ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF THE AEROBIC PATHOGENS (n=42)

TABLE 20: Gram Positive Organisms (n=23)

Organism	PEN 10µg	ERY 15µg	AK 30µg	CIP 5µg	COT 1.25/23.75 µg	CX 30µg	GM 10µg	TETR 10µg	VAN 30 µg	CL* 2µg*	HLG 120 µg
S.aureus (MSSA) (4)	100% (4)	75% (3)	75% (3)	50% (2)	50% (2)	100% (4)	75% (3)	75% (3)	NT	100% (4)	NT
S.aureus (MRSA) (13)	0% (13)	30% (4)	76.9 (10)	61.5% (8)	23.8% (3)	0% (13)	69.8% (9)	30% (4)	NT	100% (13)	NT
Streptococcus Pyogenes (3)	100% (3)	100% (3)	NT	100% (3)	NT	NT	NT	100% (3)	100% (3)	100% (3)	NT
Enterococcus faecalis(2)	100% (2)	100% (2)	NT	100% (2)	NT	NT	NT	NT	100% (2)	NT	100% (2)
Staphylococcus epidermidis (1)	100% (1)	100%(1)	100% (1)	0% (0)	0% (0)	100% (1)	100% (1)	100% (1)	100% (1)	100% (1)	NT

*Clindamycin susceptibility was reported after testing with Erythromycin for inducible resistance by D test

KEY TO THE TABLE
PEN- Penicillin, ERY- Erythromycin, AK-Amikacin, CIP-Ciprofloxicin, COT-Cotrimoxazole, CX-Cefoxitin,GM-Gentamicin,TET-Tetracycline,VAN-Vancomycin,CL-Clindamycin,HLG- High level gentamicin

ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF THE AEROBIC PATHOGENS(n=42)

TABLE 21: Gram Negative Bacilli (n=19)

Organism	AK 30µg	CIP 5µg	COT 1.25/23.75 µg	PT 100/10 µg	CAZ 30 µg	CTX 30 µg	GM 10µg	TET 10µg	IMP 10 µg	CX 30 µg
E.Coli (n=8)	75% (6)	87.5% (7)	62.5% (5)	100% (8)	25% (2)	25% (2)	75% (6)	87.5% (7)	100% (8)	100% (8)
K.oxytoca (n=2)	50% (1)	0% (0)	0% (0)	50% (1)	50% (1)	50% (1)	50% (1)	50% (1)	100% (2)	100% (2)
Proteus mirabilis (n=3)	100% (3)	100% (3)	66.6% (2)	100% (3)	100% (3)	100% (3)	66.6% (2)	33.3% (1)	100% (3)	100% (3)
Proteus vulgaris (n=1)	100% (1)	100% (1)	100% (1)	100% (1)	100% (1)	100% (1)	100% (1)	100% (1)	100% (1)	100% (1)
Pseudomonas aeruginosa (n=5)	100 % (5)	60% (3)	NT	100% (5)	100% (5)	NT	80% (1)	NT	100% (5)	100%

KEY TO THE TABLE

AK-Amikacin,CIP-Ciprofloxicin,COT-Cotrimoxazole, CX-Cefoxitin,GM-Gentamicin,TET-Tetracycline,PT-Piperacillin-Tazobactam,CAZ-Ceftazidime,CTX-Cefotaxime, IMP- Imipenam

ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF THE COLONISERS (n=77)

TABLE 22: Gram Positive Organisms (n=41)

ORGANISMS	PEN 10µg	ERY 15µg	AK 30µg	CIP 5µg	COT 1.25/ 23.75 µg	CX 30µg	GM 10µg	TET 10µg	VAN 30 µg	CL* 2µg	HLG 120 µg
S.aureus (MSSA) (20)	100% (20)	80% (16)	90% (18)	65% (13)	75% (15)	100% (20)	85% (17)	95% (19)	NT	100% (20)	NT
S.epidermidis (8)	100% (8)	100% (8)	75% (6)	75% (6)	75% (6)	100% (8)	62.5% (5)	87.5% (7)	NT	100% (8)	NT
Enterococcus faecalis (6)	100% (6)	50% (3)	NT	100% (6)	NT	NT	NT	NT	100% (6)	NT	100% (6)
Enterococcus faecium (1)	100% (1)	100% (1)	NT	0% (0)	NT	NT	NT	NT	100% (1)	NT	100% (1)
DIPHTHEROIDS (6)	100% (6)	NT	NT	100% (6)	NT	NT	NT	NT	100% (6)	NT	NT

*Clindamycin sensitivity is reported by inducible D test according to the CLSI guidelines.

KEY TO THE TABLE

PEN- Penicillin, ERY- Erythromycin, AK-Amikacin, CIP-Ciprofloxicin, COT-Cotrimoxazole, CX-Cefoxitin,GM-Gentamicin,TET-Tetracycline,VAN-Vancomycin,CL-Clindamycin,HLG- High level gentamicin

ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF THE COLONISERS (n=77)

TABLE 23: Gram Negative Bacilli (n=36)

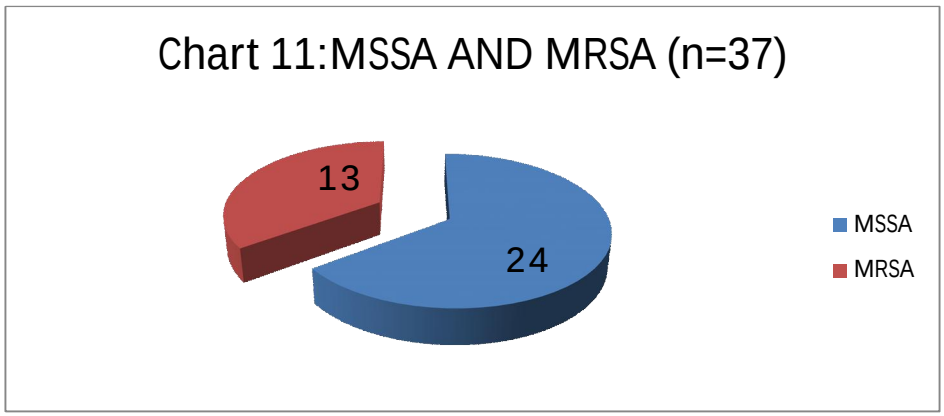
Organisms	AK 30µg	CIP 5µg	COT 1.25/23.75 µg	PT 100/10 µg	CAZ 30 µg	CTX 30 µg	GM 10µg	TET 10µg	IMP 10 µg	CX 30 µg
E.Coli (6)	100% (6)	83.3% (5)	50% (3)	100% (6)	100% (6)	100% (6)	83.3% (5)	83.3% (5)	100% (6)	100% (6)
K.oxytoca (3)	66.6% (2)	60% (3)	100% (3)	100% (3)	100% (3)	100% (3)	66.6% (2)	66.6% (2)	100% (3)	100% (3)
K.pneumoniae (3)	33.3% (1)	66.6% (2)	100% (3)	100% (3)	100% (3)	100% (3)	33.3% (1)	100% (3)	100% (3)	100% (3)
Proteus vulgaris (5)	100% (5)	60% (3)	80% (4)	100% (5)	100% (5)	100% (5)	80% (4)	80% (4)	100% (5)	100% (5)
Proteus mirabilis (7)	85.8% (6)	71.4% (5)	71.4% (5)	100% (7)	100% (7)	100% (7)	85.8% (6)	85.8% (6)	100% (7)	100% (7)
Pseudomonas aeruginosa (10)	60% (6)	60% (6)	NT	100% (10)	100% (10)	NT	50% (5)	NT	100% (10)	NT
Acinetobacter baumanii (2)	100% (2)	100% (2)	100% (2)	100% (2)	100% (2)	100% (2)	100% (2)	100% (2)	100% (2)	100% (2)

KEY TO THE TABLE

AK-Amikacin,CIP-Ciprofloxicin,COT-Cotrimoxazole, CX-Cefoxitin,GM-Gentamicin,TET-Tetracycline,PT-Piperacillin- Tazobactam,CAZ-Ceftazidime,CTX-Cefotaxime, IMP- Imipenam

TABLE 24: Methicillin Resistance Among Staphylococcus aureus Isolates By Cefoxitin Screening Method (n=37)

	No of isolates	Percentage
MSSA	24	64.84
MRSA	13	34.16
TOTAL	37	



All the isolates were susceptible to Vancomycin by MIC macrobroth dilution method.

MIC was ≤ 2µg/ml.

There was no resistance to Clindamycin by Inducible D test done according to CLSI guidelines.

Biofilm Production among MRSA isolates

Biofim formation was determined among MRSA isolates by microtitre plate method. The results were interpreted by following formula.

ODC (Optical density Cutoff) value was calculated with the formula

ODC= Mean of the Negative Control+ 3 x Standard deviation of Negative control.

ODC= 0.069

Moderate Biofilm producers had OD value above .0.207 OD

Using the given formula, Out of 13 MRSA isolates, Five were found to be Moderate Biofilm producers. Others were Weak biofilm producers

TABLE 25 : Biofilm Producers Among MRSA Isolates (n=13)

Type of Biofilm	No of cases	Percentage
Strong producers	NIL	0
Moderate biofilm producers	5	38.4
Weak Biofilm producers	8	61.5
Total	13	

TABLE 26 : Vancomycin Susceptibility Among The Methicillin Resistant Staphylococcus aureus By Macrobroth Dilution Method (n=13)

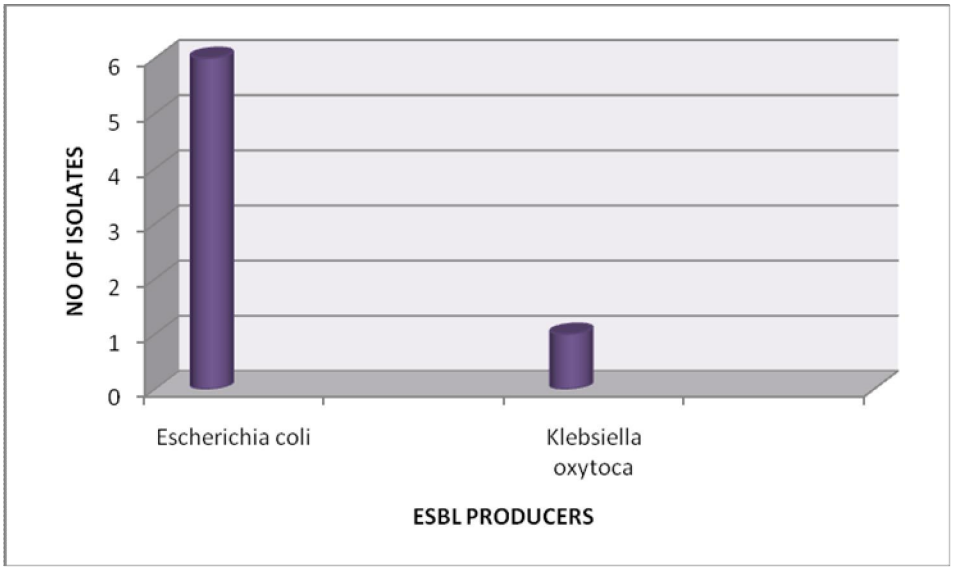
Vancomycin	MIC≤2 µG/ML	MIC 4-8 µG/ML	MIC <16 µG/ML
MRSA	13	NIL	NIL

All thirteen isolates were susceptibile to Vancomycin by MIC macrobroth dilution method.

TABLE 27: Resistant Pattern In Enterobacteriaceae Isolates (n=38)

Organism	Total no	ESBL producers	Percentage
Escherichia coli	14	6	15.7%
Klebsiella oxytoca	5	1	2.6%
Klebsiella pneumoniae	3	nil	
Proteus vulgaris	6	nil	
Proteus mirabilis	10	nil	
Total	38	7	

Chart 12: Distribution of ESBL producers (n=7)



Among the 19 Isolates of *Escherichia coli* and *Klebsiella oxytoca*, Seven were ESBL Producers by Screening Test with CTX and CAZ disk .They were confirmed by Phenotypic confirmatory test

There was no Amp C and MBL producers in this study.

DISCUSSION

This cross sectional study was conducted at the Institute of Microbiology, Madras Medical College in association with the department of General surgery,Vascular surgery, Plastic surgery, Rajiv Gandhi Government General Hospital and Madras Medical College,Chennai.

100 patients with venous leg ulcers who satisfied the inclusion criteria were included in the study.

In this study,29% of the patients were found in the age group of 61-70, followed by the 51-60 years (24%).(Table:1). Venous insufficiency progresses with age.[51]The prevalence of venous ulcer had been found to increase by 4% over the age of 65 years[5]. In the article Contemporary reviews in Cardiovascular medicine, Robert T. Eberhardt, MD; Joseph D. Raffetto, MD quote that the incidence increases with age more than fifty[52] .

Female sex is a clinically accepted risk factor for chronic venous diseases.[53] Explanation is probably that the females outlive males.[51] In our study,among 100 patients with Venous ulcer,84% were males and 16 %were females. (Table :1)

Most of the patients in this study had occupation related to standing for long hours resulting in venous hypertension[54].21% of the study population were night security workers followed by daily wagers (17%).In the article by by D. J. Radak and V. A. Sotirovic, et al on their study on risk factors for symptomatic chronic

venous disorders quote that profession related to prolonged sitting or standing was found to have increased risk for Chronic venous disease.[55](Table :4)

In the study by Dragan J. Milic, PhD, Sasa S. Zivic, et al ,the risk factor of healing was the presence of a non healing ulcer of more than 12 months .But no correlation is given for the duration of ulcer and infection rate.[56] In this study, 36% of the patients had the Venous ulcers for a period of 6-10 years (table:) but the frequency of the infection did not correlate with the duration of the ulcers. (Table :17)

Calf muscle pump dysfunction leads to venous hypertension which in turn leads to venous dysfunction and stasis of blood at lower limbs. In this study, GSV, SSV and perforator incompetence were associated with venous ulcers. 81% percent of the venous ulcers were above the medial malleolus and 43 % had perforator incompetence. In the study by Georgios Spentzouris and Nocos Labropoulos et al, the incidence of the location of ulcer above the medial malleolus was reported to be 95%[57] (Table:7).

BMI value higher than 33 kg/m^2 is quoted as a risk factor for chronic venous ulcers[56].Obesity leads to dysfunction of valves leading to poor venous return.In this study 12% of the patients were found to be obese.(p value is <0.001 which is significant) (Table :3)

Deep vein thrombosis and varicose veins leads to venous insufficiency and venous hypertension and causes venous ulcers in the lower limbs.Deep vein

thrombosis (DVT) causes damage to the valves.In the current study, 66% had visible varicose veins, 10 % of the patients had DVT and 16 % of the study population had previous surgeries associated with the venous ulcer. In the study by Dragan J. Milic, PhD,Sasa S. Zivic,et al,the incidence of DVT was 36% among the venous ulcer patients.[56] (Table:6)Smoking increases the risk for venous ulceration[58].Avoiding smoking and alcohol abstinence gives better results of compression therapy and pain management[59] In the study,25% of the study population were alcoholic and smokers.9% were smokers.(Table:5)

Venous stasis and inflammation stimulates the peripheral nerve endings Superadded infection worsens it.The increased pain is sign of infection.[6].46% of the patients in the current study had pain and 26% had discharge. 5%had malodourous discharge.In the study by Howell et al ,61% of the patients presented with increased pain[30].(Table :8).

Direct gram stain correlated with Quantitative culture in 53% of the ulcers.Direct Gram stain is a rapid indication of the bacterial burden in the wound P.G. Bowler et al states that a rapid Gram stain technique is shown to predict a microbial load of $>10^5$ CFU/g of tissue if a single microorganism is seen on the slide preparation".[26]Levine et al also concluded that the presence of bacteria in Gram stain is associated with $\geq 10^6$ bacteria or more per swab present.[60](Table :9)

Robson et al. have suggested that “quantitative bacterial counts from tissue biopsy samples of the ulcer $\geq 10^6$CFU /gram tissue indicates infection”[72][17].In this study, this criteria was followed to assess the bacterial burden in the clinically infected ulcers.According to the results of Quantitative culture,48 % of the ulcers were found to be infected.Among this,20% of ulcers had single organism isolated. 28 % of the cases had polymicrobial infection. The remaining 43% of the ulcers were considered as colonizers with either single or multiple organisms as the quantitative culture yielded < 10^6CFU/gram tissue. There was no growth in 9% of the ulcers.This is in contrast to the study conducted by Somaprakas et al,90 % of the ulcers were found to be monomicrobial[61]. In the study by Brook et al on aerobic and anaerobic microbiology of chronic venous ulcers,they concluded that chronic venous ulcers are polymicrobial with aerobic and anaerobic flora[62].More than one bacterial species were detected by Kritine et al in their study on multiple bacterial species residing in the chronic wounds.[63](Table :12)

Among the aerobes, Gram positive cocci were the predominant pathogen among the infected venous ulcers. Among the Gram positive cocci, *Staphylococcus aureus* was the commonest pathogen isolated from 17% of the ulcers followed by *Streptococcus pyogenes* in 3% of the ulcers. *Streptococcus pyogenes* infection presents with inflammation and spreads along the draining lymphatics to focal lymph nodes and rapidly through subcutaneous tissue and fascia with rapid tissue destruction[64].Madsen et al in their study on bacterial colonization and healing of venous ulcers found that ulcers with

Staphylococcus and beta haemolytic Streptococcus healed slowly.[65]*Enterococcus faecalis* are normal flora of the skin. It is implicated in wound infections. In our study, *Enterococcus faecalis* were isolated from 2% of the ulcers and *Staphylococcus epidermidis* from 1% of the venous ulcer. In study by Mustafa Fazli,et al,*Staphylococcus aureus* in 50% of the cases [66] Bowler et al in their study concluded that, *Staphylococcus aureus* was the commonest organism to be isolated[73].(Table:14)

Among the Gram negative bacilli, *Escherichia coli* was the commonest pathogen (8%) followed by *Pseudomonas aeruginosa* (5%). Brook and Frazier et al reported an isolated rate of 12% for *Escherichia coli* .[62](Table :14)

Out of 37 *Staphylococcus aureus* isolates,35.1% was *Methicillin resistant Staphylococcus aureus* (MRSA). In a study conducted in Brazil, the frequency of MRSA was 28%. [67]. It is almost impossible to eradicate MRSA from chronic wounds.[69]Howell –jones quotes that with MRSA infection, problems include cross-contamination of wounds from the patients themselves, fomites and health care personnel[30]. In the study by Frankel et al, the incidence of MRSA was 45% among patients with chronic wounds.[74] (Table:24)

Chronic wound does not heal despite adequate treatment due to the presence of biofilm. In this study, 38.4% of MRSA isolates were moderate biofilm producers. Biofilm in MRSA isolates makes them resistant to antibiotics and is due to presence of polysaccharide intracellular antigen.[61] Biofilm formation and

adherence of bacteria to host tissue are one of the most important virulence factors of *methicillin-resistant strains of Staphylococcus aureus* (MRSA).[70]In the study done in Brazil,45% of MRSA isolates from the chronic wounds were moderate biofilm producers[70] which correlated with this study.(Table :25)

30% of the MRSA isolates were susceptible to Erythromycin and all were susceptible to clindamycin by inducible D test.In the study by Martin et al Erythromycin and clindamycin resistance was 69.6% .60% of cases showed inducible clindamycin resistance[67].The absence of inducible clindamycin resistance in our study could be due to the rare usage of the drug as first line of treatment in our hospital.

All the MRSA isolates were susceptible to Vancomycin tested by determination of Minimum Inhibitory concentration by Macro broth dilution method. (Table :26)

Among the 38 isolates of Enterobactericeae, 18.3% were ESBL producers by phenotypic confirmatory method.Six isolates of Escherichia coli were ESBL producers. Among the two *Klebsiella oxytoca* isolates , one was an ESBL producer. In study conducted by Nyambura Moremi et al 41% were ESBL producers (Escherichia coli and Klebsiella species)[68]. No other significant resistance pattern was found among the pathogens in the current study. (Table:27)

Colonisers are bacteria whose multiplication causes little or no harm to the host. Though bacterial quantity and virulence define colonization, harmful or

harmless colonization depends on the local and general resistance of the host. 43% of the ulcers were found to be colonized with bacteria. 26% of the ulcer was colonized by single organism.17% were polymicrobial.

Staphylococcus aureus was the commonest organism to be isolated among colonisers in 20% of the ulcers. In the study conducted by Bowler et al on microbiology of infected and non infected leg ulcers in 1999,*Staphylococcus aureus* was the commonest organism to be isolated in non infected ulcers. (Table :15)[73]

Among the colonisers,the most common Gram negative bacilli isolated was *Pseudomonas aeruginosa* (10%) followed by *Proteus mirabilis* in 7%,*Escherichia coli* in 6%,*Acinetobacter baumanii* in 2%.Halbert et al found no significant delay in the wounds colonized by *Pseudomonas aeruginosa*.[71l] These organisms were found susceptible to all antibiotics tested. This differs from the study conducted in Eastern India, where MBL producing strain of *Acinetobacter baumanii* and *Acinetobacter lwoffi* were isolated from venous ulcer. [29]

The only significant antimicrobial resistance among colonisers was found in *Klebsiella pneumoniae* which showed 33% susceptibility to Aminoglycosides (Table :23)

The anaerobes were isolated from 14 % of the ulcers in this study. They were found in synergy with the aerobes. The tissue hypoxia caused by aerobes serves as suitable environment for anaerobes to grow[26]. *Peptostreptococcus anaerobicus* was isolated in12% of the ulcers followed by *Bacteroides fragilis in 2%* . In a

study by Brook et al on aerobic and anaerobic microbiology of chronic venous ulcers ,the anaerobes were isolated in 53.6% of cases[62]. Anaerobic isolation rate of 17% was reported in the study by Halbert et al .[71] (Table :16)

SUMMARY

- 100 venous leg ulcer patients with clinical signs of infection attending the Department of General, Plastic and Vascular surgery departments were included in the study.

- Two tissue bits were obtained from the patients for aerobic Quantitative culture and for isolation and identification of anaerobes.

- Out of 100 patients, 29 %were found in the age group 61-70 years. 84 %were males and 16% were females. Commonest anatomical location was above medial malleolus (81%). Perforator dysfunction was observed in 43% of patients. 46% of patients presented with increased pain. 36% ulcers were of 6-10 years duration.

- Risk factors associated with the ulcers were occupation, Deep vein thrombois, obesity, Varicose veins,

- Increased pain was the main presenting symptom (46%)

- Gram stain correlated with positive quantitative culture in 53% of the ulcers.

- Out of 100 ulcers, 91% of them were culture positive. 48% of the ulcers were infected.43% were colonized.9% showed no growth.Out of 48% of infected ulcers, single organism was isolated from 20 % of ulcers. 28% of the ulcers were polymicrobial.

- Gram positive cocci were the predominant pathogen in infected ulcers. *Staphylococcus aureus* was the commonest organism to be isolated in 17%of the ulcers followed by *Escherichia coli* in 8%. *Streptococcus pyogenes* was found to be the etiological agent in 3% of the ulcers

- *Methicillin resistant Staphylococcus aureus* were isolated from 13% of the ulcers. All the MRSA isolates were susceptible for Vancomycin. Out of 13 MRSA isolates, 5 were moderate biofilm producers.

- Among the 38 Enterobacteriaceae isolates, 18.3% were ESBL producers.

- Among the colonizers *Pseudomonas aeruginosa* was the commonest (10%) followed by *Proteus mirabilis* in 7%

- No significant antibiotic resistance was observed among the colonisers.

- Anaerobes were presumptively identified in 14% of the ulcers using anaerobic antibiotic identification disks. *Peptostreptococcus anaerobicus* was isolated from 12% of the ulcers and *Bacteriodes fragilis* from 2 % .

CONCLUSION

- Venous leg ulcers are predominant in the age group 61-70.
- The commonest pathology associated with venous ulcers is perforator incompetence.
- Significant risk factors associated were DVT ,Obesity and varicose veins .
- The diagnosis of infection can be done by quantitative culture method using tissue biopsy. The ulcers are monomicrobial or polymicrobial.
- Rate of infection in venous ulcers is 48%.
- *Staphylococcus aureus* is the most common pathogen followed by members of *Escherichia coli.*
- MRSA is a significant pathogen in the etiology of venous leg ulcers , majority of which were also found to be moderate biofilm producers.
- Among the Enterobacteriaceae family ,ESBL production is a cause of antimicrobial resistance though no AmpC or MBL producers were detected in this study.
- Anaerobes constitute a significant proportion of the etiological agents of the venous leg ulcer patients.

The presence of infection and non infection in the wounds should be determined for treatment purposes.As there is debate whether to treat the colonized wound Direct microscopy and quantitative microbiological culture which detects the presence of bacteria greater than 10^5 CFU/gram tissue can be an useful guide to initiate treatment of the venous leg ulcers.

FIGURE 2: VENOUS LEG ULCER

FIGURE 3: PROCESSING OF TISSUE

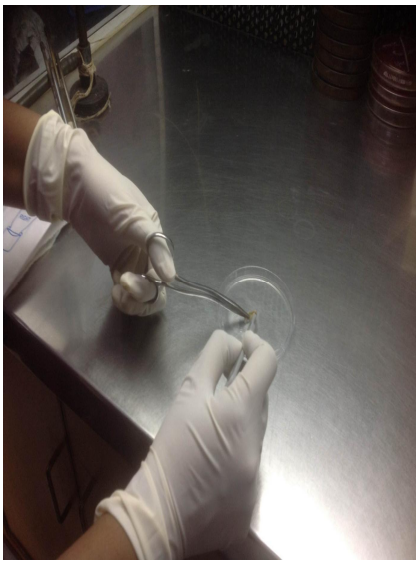


FIGURE 4: DIRECT GRAM STAIN SHOWING GRAM POSITIVE COCCI IN CLUSTERS

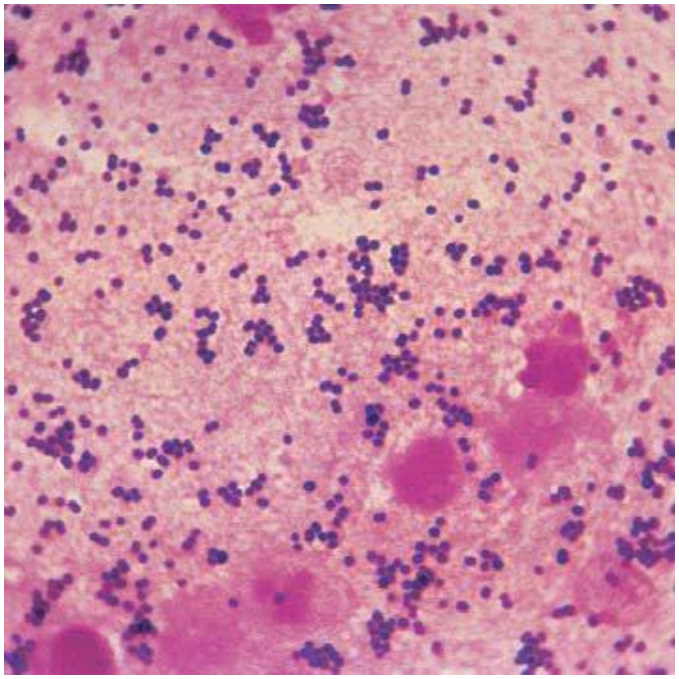


FIGURE 5: QUANTITATIVE CULTURE OF TISSUE

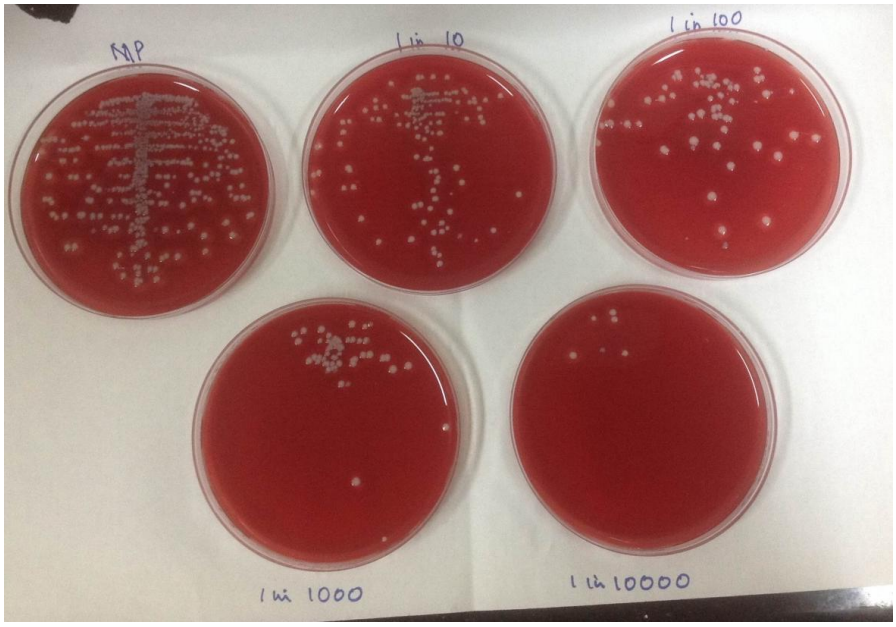


FIGURE 6: ANTIBIOGRAM OF MRSA ISOLATE SHOWING CEFOXITIN RESISTANCE

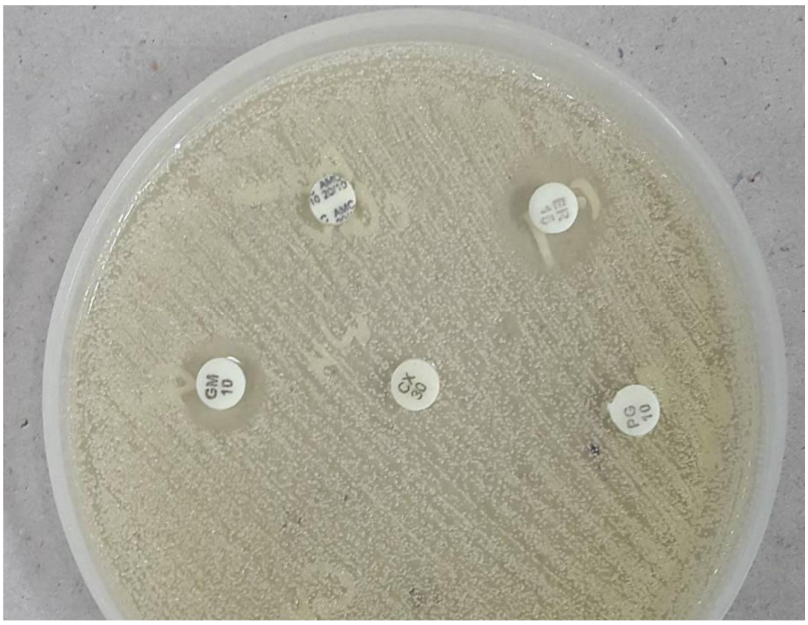


FIGURE 7: TUBE COAGULASE TEST

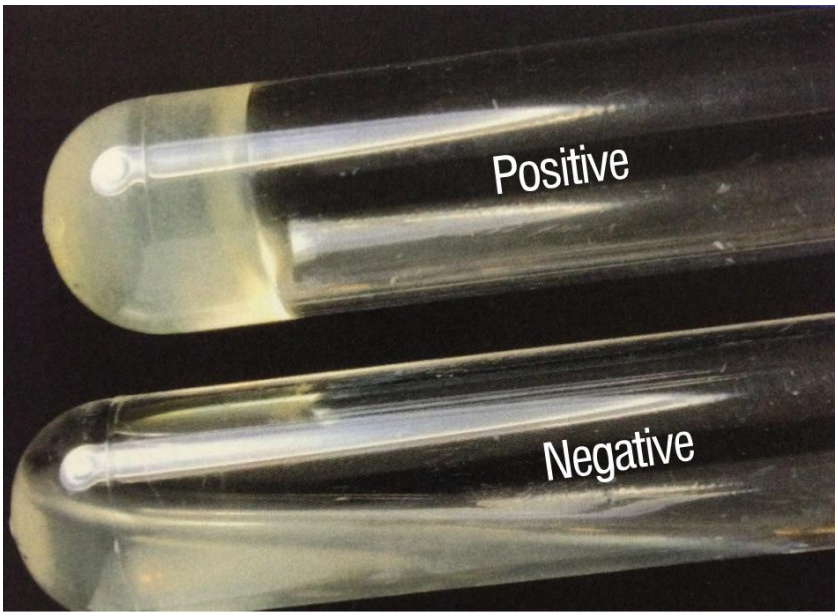


FIGURE 8: DETERMINATION OF MIC FOR VANCOMYCIN FOR MRSA ISOLATES BY MACRO BROTH DILUTION METHOD

FIGURE 9: STREPTOCOCCUS PYOGENES SHOWING BACITRACIN SENSITIVITY

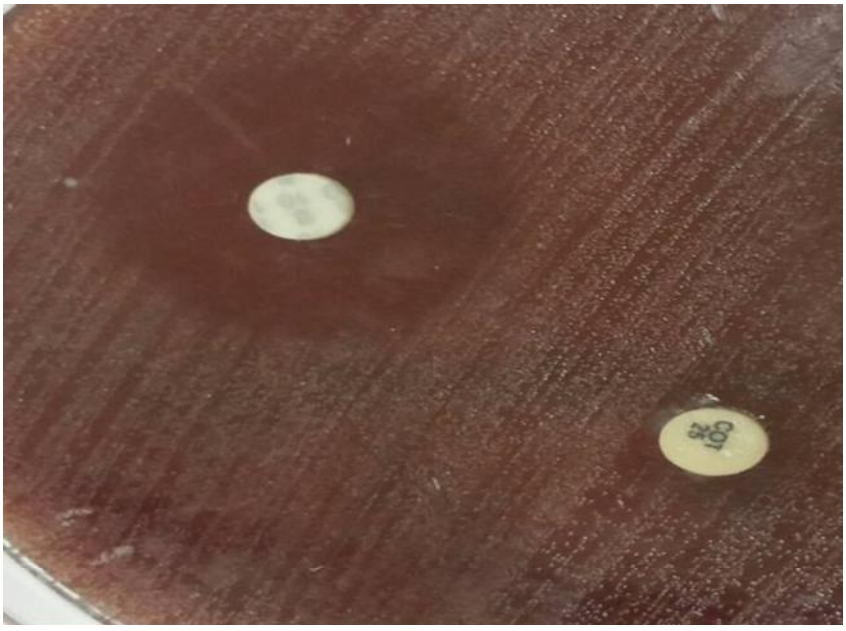


FIGURE 10: PHENOTYPIC CONFIRMATION DISC DIFFUSION TEST (PCDDT) FOR ESBL PRODUCTION

FIGURE 11: IDENTIFICATION OF ANAEROBES USING DIFFERNTIAL DISKS

FIGURE 12: GRAM STAIN FROM ANAEROBIC CULTURE SHOWING GRAM POSITIVE COCCI IN PAIRS AND SHORT CHAINS

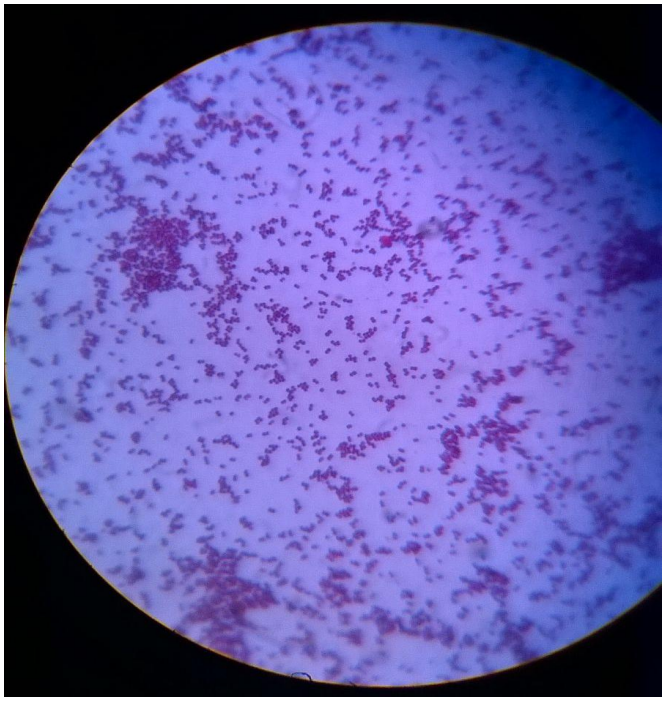
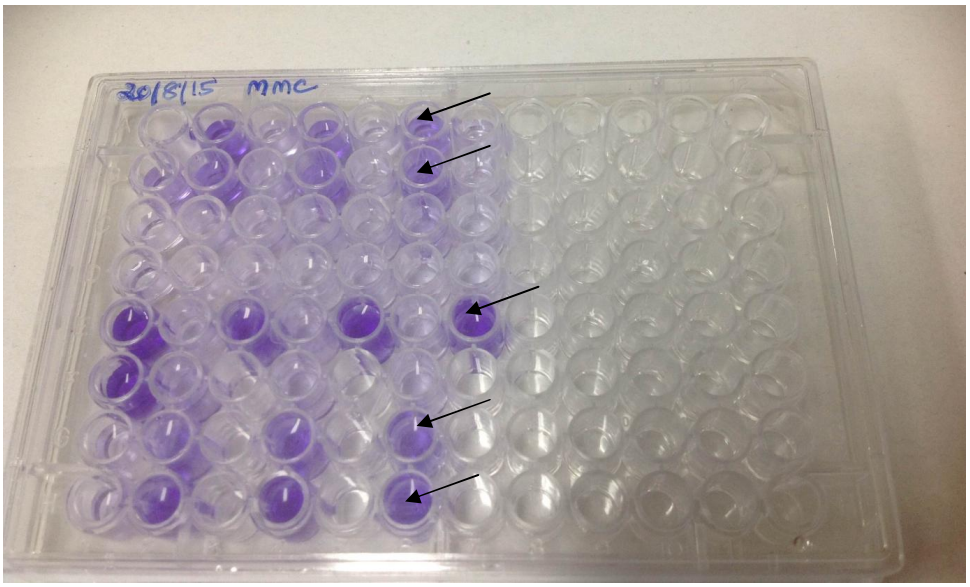


FIGURE 13: BIOFILM FORMATION BY MRSA ISOLATES



APPENDIX I

ABBREVATIONS

CVI	CHRONIC VENOUS INSUFFICIENCY
CVD	CHRONIC VENOUS DISEASE
VLU	VENOUS LEG ULCER
ESBL	EXTENDED BROAD SPECTRUM BETA LACTAMASE
MRSA	METHICILLIN RESISTANT STAPHYLOCOCCUS AUREUS
MSSA	METHICILLIN SENSITIVE STAPHYLOCOCCUS AUREUS
MBL	METALLOBETA LACTAMASE
AVF	AMERICAN VENOUS FORUM
CEAP	CLINICAL -ETIOLOGY- ANATOMY- PATHOLOGY
VSS	VENOUS SEVERITY SCORE
CFU	COLONY FORMING UNIT
IP	IN PATIENT
OP	OUT PATIENT
MIC	MINIMUM INHIBITORY CONCENTRATION
CLSI	CENTRAL LABORATORY STANDARD INSTITUTE
BSAC	BRITISH SOCIETY OF ANTIBIOTIC CHEMOTHERAPY
VSI	VASCULAR SOCIETY OF INDIA
VAI	VENOUS ASSOCIATION OF INDIA
DVT	DEEP VEIN THROMBOSIS
GSV	GREAT SAPHENOUS VEIN
SSV	SHORT SAPHENOUS VEIN

IVC	INFERIOR VENACAVA
VEGF	VASCULAR ENDOTHELIAL GROWTH FACTOR
TGF	TRANSFORMING GROWTH FACTOR
MMP	MATRIX METALLOPROTEINASES
EMMPRIN	EXTRACELLULAR MMP INDUCER
VCSS	VENOUS CLINICAL SEVERITY SCORE
VSDS	VENOUS SEGMENTAL DISEASE SCORE
VDS	VENOUS DISABILITY SCORE
ABPI	ANKLE BRACHIAL PRESSURE INDEX
TBPI	TOE BRACHIAL PRESSURE INDEX
ATCC	AMERICAN TYPE CULTURE COLLECTION
MR	METHYL RED
VP	VOGES PROSKAUER
CM	CENTI METERS
MM	MILLI METERS
GM	GRAMS

APPENDIX II

A. STAINS AND REAGENTS

1. Gram staining

Methyl violet (2%)	l0g Methyl violet in 100ml absolute alcohol
	in 1 litre of distilled water (primary stain)
Grams Iodine	l0g Iodine in 20g KI (fixative)
Acetone	Decolourising agent
Carbol fuchsin 1%	Secondary stain.

B. MEDIA USED

Mac Conkey agar

Peptone	20g
Sodium taurocholate	5g
Distilled Water	1 ltr
Agar	20 g
2% neutral red in 50% ethanol	3.5ml
10% lactose solution	l00ml

Dissolve peptone and taurocholate in water by heating. Add agar and dissolve it in steamer. Adjust pH to 7.5. Add lactose and neutral red shake well and mix.

Heat in free steam (100℃) for 1 hour, then autoclave at 115℃ for 15 minutes.

2. Nutrient agar

Peptic digest of animal tissue	5g
Sodium chloride	5g
Beef extract	1.5g

Yeast extract	1.5g
Agar	15gm

Final pH 7.4±0.2

Suspend 28 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely and sterilized by autoclaving at 15 lbs pressure (120°C) for 15 minutes.

3. Blood agar (5% sheep blood agar)

Peptone	l0g
NaCl	5g
Distilled water	1 Ltr
Agar	l0g

Dissolve ingredients in distilled water by boiling, and add 5% sheep blood (sterile) at 55°C adjust pH to 7.4.

4. Chocolate agar

Sterile defibrinated blood	10 ml
Nutrient Agar (melted)	100 ml

When the temperature was about 75°C, sterile blood was added with constant agitation. After addition of blood, kept in water bath and heating was continued till the blood changed to chocolate colour. Cooled to about 50° C and poured about 15ml into petri dishes with sterile precaution.

5. **Cation adjusted Mueller- Hinton Agar**

Beef infusion	300ml
Caesein hydrolysate	17.5g

Starch	1.5g
Agar	l0g
Distilled water	1 ltr

pH = 7.4

Sterilise by autoclaving at 121°C for 20 mins

6. Robertson's Cooked Meat Broth

Fresh bullock heart	500g
Water	500ml
Sodium hydroxide, mol /Lit	1.5ml
Liquid filtered from cooked meat	500ml
Peptone	2.5g
NaCl	1.25g

7. Selective Anaerobic Blood Agar:

1 µg/ml menadione and 20 µg/ml gentamicin added to the blood agar.

8. Thioglycollate broth

Pancreatic digest of casein	15gms
Yeast extract	5gms
Dextrose (Glucose)	5.5gms
Sodium chloride	2.5gms
L-Cystine	0.5gms

Autoclaved at 15 lbs pressure (121°C) for 20 minutes.

Note: If more than the upper one-third of the medium has acquired a pink colour, the medium may be restored once by heating in a water bath or until the pink colour disappears.

C. MEDIA REQUIRED FOR BIOCHEMICAL IDENTIFICATION

1. Oxidase Reagent

Tetra methyl p-phenylene diamine dihyrochloride- 1% aqueous solution.

2. Catalase

3% hydrogen peroxide

3. Coagulase test

Tube coagulase test

1. Prepare a 1 in 6 dilution of the plasma in saline and place 1 ml volume of the diluted plasma in small tube.
2. Emulsify a colony of the Staphylococcus under test in the tube of the diluted plasma
3. Appropriate controls were put up
4. Incubate the tubes at 37°Cfor 4 hours
5. Examine the tubes at 1,2,3 4 hours for Clot formation
6. Leave the tubes at room temperature overnight and reexamine
7. Read as positive any degree of clot formation

Slide Coagulase test

Emulsify a staphylococcus colony in a drop of water on a microscope slide with a minimum of spreading .Make a similar suspensions of control positive and negative strains to confirm the proper reactivity of the plasma. Stir the adhering

plasma into Staphylococcal suspension on the slide. Formation of Clumps are read as Positive.

4. Indole test

Kovac's reagent

Amyl or isoamyl alcohol 150ml Para dimethyl amino benzaldehyde – l0g

Concentrated hydrochloric acid - 50ml

Dissolve the aldehyde in the alcohol and slowly add the acid. Prepare in small quantities and store in the refrigerator. Shake gently before use.

5.Christensen's Urease test medium

Peptone	lg
Sodium chloride	5g
Dipotassium hydrogen phosphate	2g
Phenol red	6ml
Agar	20g
Distilled water	1 ltr
10% sterile solution of glucose	10ml
Sterile 20% urea solution	100ml

Sterilize the glucose and urea solutions by filtration. Prepare the basal medium without glucose and urea, adjust to pH 6.8-6.9 and sterilize by autoclaving in a flask at 121℃ for 30min. Cool to about 50°C, add the glucose & urea, and tube the medium as slopes.

6. Simmon's Citrate Medium

Koser's medium	1 ltr
Agar	20 g
Bromothymol blue 0.2%	40ml

Dispense, autoclave at 121°C for 15 min and allow to set as slopes

7. Triple Sugar Iron medium

Beef extract	3g
Yeast extract	3g
Peptone	20g
Glucose	lg
Lactose	10 g
Sucrose	l0g
Ferric citrate	0.3g
Sodium chloride	5g
Sodum thiosulphate	0.3g
Agar	12g
Phenol red 0.2% solution	12ml
Distilled water	1 ltr

Heat to dissolve the solids, add the indicator solution, mix and tube. Sterilize at 121°C for 15 min and cool to form slopes with deep butts.

8. Glucose phosphate broth

Peptone	5g
Dipotassium hydrogen phosphate	5g
Water	1 ltr
Glucose 10% solution	50ml

Dissolve the peptone and phosphate and adjust the pH to 7.6. Filter dispense in 5ml amounts and sterilize at 121°C for 15min. Sterilize the glucose solution by filtration and add 0.25ml to each tube.

Methyl Red Reagent

Methyl Red	l0mg
Ethyl alcohol	30ml
Distilled water	20ml

VogesProskauer Reagent

Reagent A: Alpha naphthol	5g
Ethyl alcohol	100ml
Reagent B: Potassium hydroxide	40g
Distilled water	100ml

9. Peptone water fermentation test medium

To the basal medium of peptone water, add sterilised sugars of 1% indicator bromothymol blue with Durham's tube. Basal medium peptone water Sugar solutions:

Sugar	1ml	
Dislilled water	100ml	pH = 7.6.

10.Mannitol motility medium

Agar	5g
Peptone	lg
Potassium nitrate	1g
Mannitol	2g
Phenol red indicator	
Distilled water	1000ml
pH	7.2

12. Potassium nitrate broth

Potassium nitrate (KNO3)	0.2gm
Peptone	5.0gm
Distilled water	100ml

The above ingredients were mixed and transferred into tubes in 5 ml amount and autoclaved.

13. Phenyl alanine deaminase test

Yeast Extract	3g
Dl-Phenylalamine	2 g
Disodium hydrogen phosphate	l g
Sodium Chloride	5 g
Agar	12g
Distilled water	1 lr
PH	7.4

Distributed in tubes and sterilized by autoclaving at 121° C for 1 5 minutes, allowed to solidify as long slopes.

14. Sugar fermentation medium

Peptone	15g
Andrade's indicator	10 ml
Sugar to be tested	20g
Water	1 litre

Andrade's indicator is prepared from 0.5% aqueous acid fuchsin to which sufficient 1M sodium hydroxide has been added to turn the colour of the solution yellow.

Dissolve the peptone and Andrade's indicator in 1 litre of water and add 20g of the sugar; sugars to be tested generally include glucose, sucrose, lactose and maltose. Distribute 3ml amounts in standard test tubes containing an inverted Durham tube. Sterilize by steaming at 100 degree C for 30 min on 3 consecutive days.

APPENDIX III

Panel of antibiotic Disk used for *Staphylococcus aureus and Coagulase negative Staphylococcus* (Hi Media)

ANTIBIOTIC DISK	CONENT	SENSITIVE (mm)	INTERMEDIATE (mm)	RESISTANT (mm)
Penicillin	10 units	≥29	-	≤28
Cefoxitin	30µg	≥22(For S.aureus and S.lugdenesis)	-	≤21
	CoNS	≥25	-	≤24
Gentamicin	10µg	≥15	13-14	≤12
Amikacin	30µg	≥17	15-16	≤14
Tetracycline	30µg	≥19	15-18	≤14
Ciprofloxacin	5µg	≥21	16-20	≤15
Erythromycin	15µg	≥18	14-17	≤13
Clindamycin	2µg	≥21	15-20	≤14
Trimethoprim-Sulfamethoxazole	1.25/23.75µg	≥16	11-15	≤10
Linezolid	30µg	≥21	-	≤20

Panel of drugs used for *Enterococcus spp*

Antibiotics	Contents	Sensitive (mm)	Intermediate (mm)	Resistant (mm)
Pencillin	10 units	≥15	-	≤14
Vancomycin	30µg	≥17	15-16	≤14
Tetracycline	30	≥19	15-18	≤14
Erythromycin	15µg	≥23	14-22	≤13
Ciprofloxacin	5µg	≥21	16-20	≤15
High level gentamicin	120µg	≥10	7-9	≤6
Chloramphenicol	30µg	≥18	13-17	≤12

Panel of drugs used for *β Hemolytic streptococcus spp*

Antibiotic	Content	Sensitive(mm)	Intermediate(mm)	Resistant(mm)
Pencillin	10 units	≥24	-	-
Cefotaxime	30µg	≥24	-	-
Vancomycin	30µg	≥17	-	-
Erythromycin	15 µg	≥21	16-20	≤15
Tetracycline	30 µg	≥23	19-22	≤18
Ofloxacin	5 µg	≥16	13-15	≤12
Clindamycin	2 µg	≥19	16-18	≤15

Panel of drugs used for Diphtheroids (According to BSAC Guidelines)

Antibiotic	Content	Sensitive(mm)	Intermediate(mm)	resistant(mm)
Penicillin	10 µg	20		19
Ciprofloxicin	5 µg	17	12-16	11
Vancomycin	30 µg	20		19

Panel of drugs used for Enterobacteriaceae

Antibiotic	Content	Sensitive(mm)	Intermediate(mm)	resistant (mm)
Cefotaxime	30 µg	≥26	23-25	≤22
Ceftazidime	30 µg	≥21	18-20	≤17
Gentamicin	10 µg	≥15	13-14	≤12
Amikacin	30 µg	≥17	15-16	≤14
Tetracycline	30 µg	≥15	12-14	≤11
Ciprofloxacin	5 µg	≥21	16-20	≤15
Trimethoprim-Sulfamethoxazole	1.25/23.75 µg	≥16	11-15	≤10
Chloramphenicol	30 µg	≥18	13-17	≤12
Imipenam	10 µg	≥23	20-22	≤19
Cefoxitin	30 µg	≥18	15-17	≤14
Piperacillin-tazobactam	100/10 µg	≥21	18-20	≤17

Panel of drugs used for *Pseudomonas aeruginosa*

Antibiotic	Disk Content	Sensitive(mm)	Intermediate(mm)	Resistant(mm)
Piperacillin-tazobactam	100/10 µg	≥21	15-20	≤14
Ceftazidime	30µg	≥18	15-17	≤14
Imipenam	10µg	≥19	16-18	≤15
Amikacin	30µg	≥17	15-16	≤14
Ciprofloxacin	5µg	≥21	16-20	≤15
Gentamicin	10µg	≥15	13-14	≤12

Panel of drugs used for *Acinetobacter spp*

Antibiotic	Disk Content	Sensitive (mm)	Intermediate (mm)	Resistant (mm)
Piperacillin-tazobactam	100/10 µg	≥21	15-20	≤14
Ceftazidime	30µg	≥18	15-17	≤14
Cefotaxime	30µg	≥23	15-22	≤14
Imepenam	10µg	≥22	19-21	≤18
Gentamicin	10µg	≥15	13-14	≤12
Amikacin	30µg	≥17	15-16	≤14
Tetracycline	30µg	≥15	12-14	≤11
Ciprofloxacin	5µg	≥21	16-20	≤15
Trimethoprim-Sulfamethoxazole	1.25/23.75 µg	≥16	11-15	≤10

ANNEXURE 1

CERTIFICTE OF APPROVAL

INSTITUTIONAL ETHICS COMMITTEE
MADRAS MEDICAL COLLEGE, CHENNAI-3

EC Reg No.ECR/270/Inst./TN/2013
Telephone No. 044 25305301
Fax : 044 25363970

CERTIFICATE OF APPROVAL

To
Dr. G.Banumathi Rathika,
Postgraduate M.D.(Microbiology),
Madras Medical College,
Chennai – 600 003.

Dear Dr.G.Banumathi Rathika,

The Institutional Ethics Committee has considered your request and approved your study titled **"A study on secondary bacterial infection in venous leg ulcer patients and their antimicrobial susceptibility pattern in a tertiary care hosspital ". No.12112014.**

The following members of Ethics Committee were present in the meeting held on 11.11.2014 conducted at Madras Medical College, Chennai-3.

13. Dr.C.Rajendran, M.D.,	: Chairperson
14. Dr.R.Vimala, M.D., Dean, MMC, Ch-3	: Deputy Chairperson
15. Prof.B.Kalaiselvi, M.D., Vice-Principal, MMC, Ch-3	: Member Secretary
16. Prof.R.Nandini, M.D., Inst.of Pharmacology, MMC	: Member
17. Prof.P.Ragumani, M.S., Professor, Inst.of Surgery, MMC	: Member
18. Prof.Md.Ali, M.D., D.M., Prof. & HOD of Medl.G.E., MMC	: Member
19. Prof.K.Ramadevi, Director i/c, Inst.of Biochemistry, MMC	: Member
20. Prof.Saraswathy, M.D., Director, Pathology, MMC, Ch-3	: Member
21. Prof.S.G.Sivachidambaram, M.D., Director i/c, Inst.of Internal Medicine, MMC	: Member
22. Thiru S.Rameshkumar, Administrative Officer	: Lay Person
23. Thiru S.Govindasamy, B.A., B.L.,	: Lawyer
24. Tmt.Arnold Saulina, M.A., MSW.,	: Social Scientist

We approve the proposal to be conducted in its presented form.

The Institutional Ethics Committee expects to be informed about the progress of the study and SAE occurring in the course of the study, any changes in the protocol and patients information/informed consent and asks to be provided a copy of the final report.

MEMBER SECRETARY
INSTITUTIONAL ETHICS COMMITTEE
MADRAS MEDICAL COLLEGE
CHENNAI-600 003

Member Secretary, Ethics Committee

ANNEXURE –II

PROFORMA

- Name : OP NO/IP NO:
- Age: Ward:
- Sex:
- Occupation:
- Address:

Presenting complaints:
Site of the Ulcer:
Duration of Ulcer:
Past history:

Personal history:

- Alcohol intake:
- Cigarette smoking:

Associated factors:

- Pregnancy
- Known tuberculosis patient /HIV
- Chronic steroid intake
- Diabetes mellitus
- Lymphoma /Leukemia /Malignancy/on Chemotherapy
- Previous Surgeries:
- Hypertension
- Any Other Abdominal Tumours

•General examination:

Built:

Weight:

Nourishment:

BP:

Temperature:

CVS:

RS:

Abdomen:

Local examination

Any Visible Varicose Veins:

Skin changes:

Site of Ulcer

Signs of Inflammation

Redness:

Odour:

Cellulitis:

Provisional diagnosis:

Laboratory evaluation:

Biochemical parameters:

- Plasma glucose levels
- Blood urea
- Creatinine

Hematological investigations:

- TC
- DC
- Hb estimation
- ESR

Doppler study :

Microbiological investigation:

Sample collected:

- Tissue bits from the Ulcer

Direct examination:

- Gram’stain:

Bacterial Culture: Tissue

Aerobic:

- MAC
- BAP

Anaerobic

- BAP
- CAP

Colony Count

Antibacterial susceptibility pattern:

ANNEXURE III

INFORMATION SHEET

STUDY TITLE: "A study on secondary bacterial infections in patients with venous leg ulcer and their antimicrobial susceptibility pattern in a Tertiary care Hospital"

Venous leg ulcer (stasis ulcers, varicose ulcers) is a severe clinical manifestation of chronic venous insufficiency (CVI). The healing of venous ulcers is a complex process, which requires a multifaceted approach, including compression, debridement, and control of bacterial overgrowth. Bacterial colonization and the growth of bacteria on Venous Ulcers occurs since it is a moist surface . Microbial infection delays the healing of the Venous Ulcer and Complicates the Treatment. These Microorganisms develop anti-microbial resistance and are difficult to treat with antimicrobial agents. I am going to evaluate the organism infecting the Venous Ulcer and determine the antibiotic susceptibility pattern. I am going to collect Pus samples and Tissue samples from patients who have Venous Ulcer and process them accordingly.100 patients are included in this study after getting informed consent only. This study is entirely voluntary and patient can withdraw any time from this study. Extra cost will not be incurred to the patients in this study. Any doubt regarding this study will be willingly clarified. Results of the study will be published. In case of any doubt.

Signature of investigator Signature of Participant

Date:

PATIENT CONSENT FORM

TITLE OF THE STUDY : "A study on secondary bacterial infections in patients with venous leg ulcer and their antimicrobial susceptibility pattern in a Tertiary care Hospital"

Name : Date :

Age : OP No :

Sex : Project Patient No:

Documentation of the informed consent

I _____ have read the information in this form (or it has been read to me). I was free to ask any questions and they have been answered. I hereby give my consent to be included as a participant in "**A study on secondary bacterial infections in patients with venous leg ulcer and their antimicrobial susceptibility pattern in a Tertiary care Hospital''**
I have read and understood this consent form and the information provided to me.

1. I have had the consent document explained to me.
2. I have been explained about the nature of the study.
3. I have been explained about my rights and responsibilities by the investigator.
4. I have been informed the investigator of all the treatments I am taking or have taken in the past _____ months including any native (alternative) treatment.
5. I have been advised about the risks associated with my participation in this study.
6. I agree to cooperate with the investigator and I will inform him/her immediately if I suffer unusual symptoms.
7. I have not participated in any research study within the past _____ month(s).
8. I am aware of the fact that I can opt out of the study at any time without having to give my reason and this will not affect my future treatment in this hospital.
9. I am also aware that the investigator may terminate my participation in the study at any time, for any reason, without any consent.
10. I hereby give permission to the investigators to release the information obtained from me as result of participation in this study to the sponsors, regulatory authorities, Govt. agencies, and IEC. I understand that they are publicly presented.

11. I have understand that my identity will be kept confidential if my data are publicly presented.
12. I have had my questions answered to my satisfaction.
13. I have decided to be in the research study.

I am aware that if I have any question during this study, I should contact the investigator. By signing this consent form I attest that the information given in this document has been clearly explained to me and understood by me, I will be given a copy of this consent document.

For participants:

Name and signature / thumb impression of the participant (or legal representative if participant incompetent/For age 10-17 yrs-Name& signature of the parent/guardian.)

Name _____
Signature_____

Date_____

Name and Signature of impartial witness (required for illiterate patients):

Name _____
Signature_____

Date_____

Address and contact number of the impartial witness:

Name and Signature of the investigator or his representative obtaining consent:

Name _____
Signature_____

Date_____

OP NO/IPNO	AGE	SEX	OCCUPATION	MAIN C/O	DURATION OF ULCER	SIDE	SITE OF ULCER	DM/HT/CO	surgery for varicose veins	ALCOHOLIC/SMOKER	VISIBLE VA	INVESTIGATIONS-DOPPLER	OTHER INVESTIGATIONS
IP no 11725	50	M	Cycle Token worker	pain	10 years	Rt	above Rt medial malleolus	DM	OPERATED FOR VARICOSE VEINS 3 TIMES	ALCOHOLIC/SMOKER	NIL	Rt-SFV junction incompetence grade I	TC-7200,DC-P70,L-30 HB-14.1,ESR-2/8,RBS-116
op no:1314277	65	M	Security worker	pain	8 years	Lt	above 2cms Lt-Medial malleolus	DM		ALCOHOLIC	YES	Lt SFV junction incompetence grade II	TC-7260,DC-P-65,L-32,E-3, HB-15.2,ESR-4/8,F-206,PP-300,Bl urea-32,Creatin
op no:26456	53	M	Security worker	pain	8years	Lt	Lt-Above lateral Malleolus	OPERATED FOR Haemorrhoidectomy 10 years,Total Hip replaceme		NIL	YES	Lt-Partial DVT of Popliteal, Post Tibial veins with dilatation of SF	TC-5800,DC-P70,L-28,E-2,Hb-15.2,ESR-4/8,RBS-86 urea-30,creatinine-0.9
op no:11950	48	M	Vegetable vendor	discharge	10 years	Rt	Below Rt Lateral Malleolus	DM		Alcoholic	YES	Rt-SFV junction incompetence grade II	TC-14000,DC P-90,L6,E4,HB-14.4,ESR-40/80
ip no 141322	47	M	Cook	swelling	2 years	Lt	Lt-Above medial Malleolus	NIL		ALCOHOLIC/SMOKER	B/L Varicos	B/L sapheno femoral junction incompetence Grade I	TC-9000,DC P-70,L-30 HB-14.1,ESR-2/8
opno:VS-1235/15	70	M	Security worker	pain	10 years	Rt	above Rt medial malleolus	NIL	Operated two times for varicose veins	ALCOHOLIC/SMOKER	YES	Rt-SFV junction incompetence grade II	TC-5400,DC-P50,L-45,E-5,Hb-13.8,ESR-6/8,RBS-98, BU-28,CREATININE-0.9
op no:632/15	65	M	Security worker	pain	8 years	Lt	Above Lt medial malleolus	DM/HT		NIL	YES	Lt-SFV junction incompetence grade II	TC-7800;DC-P60,L-38,E 2;HB-14.8,ESR 4/8 RBS-160, Bl UREA 6 CREATITINE 1.
op no 6275	62	M	COOK	pain	10 years	Lt	ABOVE LT ANKLE-lateral malleolus	DM	OPERATEDFOR VARICOSE VEINS 2 YEARS	NIL	NIL	Lt-sfv junction incompetence grade I,below knee perforator inco	TC-11OOO,DC-P74,L26,HB-6.7,ESR 70,RBS -273,UREA 36,CREATININE1.2
op no 67081	48	M	Electrician	pain	4 years	Lt	Lt-Above medial Malleolus	NIL		NIL	NIL	Lt SFV junction incompetence grade I,B/Knee,15 cms above ankl	TC-7200,DC-P42,L-56,E-2 HB-15.7,ESR-2/8,RBS-108, UREA 26,CREATININE1.0
opno:VS-1962/13	60	M	Tea stall owner	pain	5 years	Rt	2 cms above Rt Medial malleolus	OBESE		ALCOHOLIC	YES	Rt-sfv junction incompetence grade II	TC- -7200,DC-P60,L9,E1,HB-16.2
ip no 7826/wd 30s	60	M	Tea stall owner	swelling	7 YEARS	Rt	above Rt medial malleolus	NIL		ALCOHOLIC/SMOKER	YES	RT-SFV JUNCTION INCOPETENCE ,NO EVIDENCE OF THROMBOSI	TC-13900,DC-P76,L16,M7,E1,HB-9.2,ESR 70,RBS-118,BLOOD UREA 45 CREAT
op no:2611/14	35	M	Hotel server	pain	5years	Lt	Above Lt medial malleolus	OBESE		ALCOHOLIC/SMOKER	NIL	Lt- Knee perforator incompetence	TC-5200;DC-P-68,L-28,E-6,HB-15.2,ESR 2/8 RBS-98,BLOOD UREA-32,CREATIN
op no 8272/14	45	M	Farmer	discharge	8 years	Lt	above Lt medial malleolus extending to lateral asce	NIL	operated for Varicose Veins 2 years back	ALCOHOLIC	NIL	POSTPHLEBHOTOMY STATUS LT KNEE PERFORATOR INCOMPETENCE	
op no :872/14	38	M	Tailor	pain	5 years	Rt	GAITER REGION	NIL	Skin grafting done -2 yrs back ,Previous DVT 2 years ba	NIL	NIL	Rt-SFJ incompetence Grade I	TC-8200,DC-P-72,L-26,E-2,HB-13.2 GMS,ESR-20/40
op no 7562	60	F	house wife	Pain/discharge	6 years	Lt	Lt-Above medial Malleolus	HT/CVA R		NIL	NIL	Lt-SFV junction incompetence	TC-4500,DC-P70,L29,E-1,HB-7.2 GRAMS,ESR-70
op no:636/13	67	M	iron man	discharge	7 years	Rt	Rt-above medial malleolus	NIL		NIL	YES,DILATE	Rt-SFJ incompetence Grade III	TC-5200;DC-P-56,L-32,E-6,HB-13.2,ESR 8/16 RBS-120,BLOOD UREA-27.7,CRE
op no;185659	45	M	Barber	Pain	8 years	Lt	Rt-below ankle joint	NIL		ALCOHOLIC	NIL	Lt-SFV INCOMPETENCE GRADE I	TC-7800;DC-P68,L-26,E 6;HB-15.8,ESR 4/8 RBS-92, BI UREA286 CREATITINE 0
op no;889/13	47	M	Autodriver	swelling	5 years	Rt	Below Rt knee and above Rt-medial malleolus	NIL		ALCOHOLIC	yes	Rt-SFV Incompetence above knee and below knee perforator in	TC-9200,DC P-68,L-28,E-4 HB-16.2,ESR-6/10 RBS-120,UREA-22 CREATININE-1
op no:363/14	40	M	worker in plastic company	Pain	6months	Rt	Rt-above medial malleolus	HERNIORRHAPHY -8 MONTHS BACK,OBESE		ALCOHOLIC/SMOKER	YES	Rt-SVJ Incompetence	TC-5600,DC-P-70,L-0,HB-15.0GMS,ESR2/8,RBS-128 UREA-30,CREATININE0.8
op no:37236	35	M	Gardener	pain	8 years	Lt	Lt-Above medial Malleolus	DM		ALCOHOLIC		Echogenous thrombosis noted ext CFV upto Popliteal vein occlu	TC-9800,DC-P-76,L24,HB-12.8 GMS,ESR-12/18,RBS108,UREA-36,CR-1.1,
op no:12900	39	M	Factory worker	Pain	8 years	Rt	Rt-above medial malleolus	HT		ALCOHOLIC/SMOKER	yes	Rt-SFV Incompetence	TC-8200 DC-P 64 L6,HB-15.2 ESR 2/8 RBS 128
op no:46879	66	M	watchman	Pain	5 years	Rt	Rt-above medial malleolus			ALCOHOLIC/SMOKER	YES	B/L sapheno femoral junction incompetence Grade II	TC-5400,DC-P54,L-36,E-8,M-2,Hb-13.2,ESR-2/8,RBS-98, BU-32,CREATININE-0
op no:44954	65	F	house wife	Pain/discharge	8 years	Rt	Rt-above medial malleolus	DM/DVT -3 years back			YES	B/L BK,AK,PERFORATORINCOMPETENCE	TC-7500,DC-P72,L28,HB-9.2 ,ESR 16/2,RBS -220 UREA-36 CR-1.2
opno:370074	67	M	Farmer	Pain/discharge	10 years	Lt	On lateral malleolus near ankle joint	NIL	Nil	ALCOHOLIC/SMOKER	NIL	B/L BK,AK,PERFORATOR INCOMPETENCE LT SIDE	TC-7200,DC-P72,L28,HB-15.2 ,ESR 2/8 RBS 108 UREA-30 CR-0.8
op no:4090/09	56	M	watchman	Pain	5 yeasr	Rt	Rt-above medial malleolus	NIL		ALCOHOLIC/SMOKER	NIL	Rt-SFV junction incompetence gradeII	TC-5500,DC P-64,L-36,ESR 4/8,HB-12.2 ,RBS 122 UR-30,CR-0.8
op no.715/94	68	M	watchman	Pain/discharge	20 years	Lt	Lt lateral malleolus	DM	NIL	ALCOHOLIC/SMOKER	Yes	Lt-SFV junction incompetence Grade II	TC- , 7800, DC-P54, L-36, E-8. HB-15.2,ESR-2/8 RBS-180, Urea-32,CR-1.1
op no2556/09	65	M	Security worker	Pain	5years	Rt	Rt-above medial malleolus	NIL		SMOKER	nil	Rt-SFJ incompetence	TC-6200,DC P-68,L 32 HB-14.1,ESR 2/8 RBS -90 UR-30 CR-0.8
op no 256/15	45	F	house wife	Pain	6 years	Rt>lt	Rt-above medial malleolus	NIL	Nil	NIL	YES	B/L SVJUNCTION INCOMPETENCE GRADE I RT-BELOW KNEE PER	TC-8000,DC P70,L0,HB-10.2 ESR-8/16,R 98, UR-, CR-0.9
op no127/15	68	M	Aavin worker (Retd)	Pain/discharge	15 years	Lt	Lt-Above medial Malleolus	NIL		SMOKER	NIL	Lt-sfv junction incompetence grade III	TC-7800,DC P-62,L37,E-1,HB-15.2, ESR 2/8,RBS 98,UR-30,CR-0.8
op no: 165/09	38	M	Coolie	Pain/discharge	6 years	Rt	lateral malleolus	DM		NIL	NIL	Rt-SFJ I/C AK ,BK,PERFORATOR I/C LT-SFV JUNCTION COMPETEN	TC-10000,DC-P-80,L-20,HB-10.2,ESR-16/32,RBS 150,UR-30,CR-1.0
op ni : 29/2015	49	M	iron man	pain	10 years	Lt	Multiple ulcer Lt- side of leg dorsal ascept	NIL	Nil	NIL	NIL	Lt-SFJ INCOMPETENCE GRADE II	TC-4800,DC-P-64,L32,E4 HB-15.2,ESR -8/16,RBS-98,UR-22,CR-0.8
op no: 335/15	38	M	coolie	discharge	10 years	Lt	Lt-Above medial Malleolus			Alcoholic	NIL	Lt-SFV junction incompetence with grade i	TC-7200, DC-P-70,L-26,E-4,HB -14.2,ESR-2/8,RBS 92,UR-24,CR-0.8
op no;2382/04	25	M	Centering Coolie	Cellulitis	5 years	lt	Lt-Above medial Malleolus			Alcoholic	yes	Lt-SFV junction I/C grade I	TC-4200,DC-P 72,L26,E-,HB-15.2,ESR 2/8,RBS-92,UR-19,CR-1.0
op no:4425/08	55	F	house wife	pain	10 years	Rt	Rt-above medial malleolus	NIL	Nil	NIL	YES	Rt-SVJ Incompetence grade I	TC-6200,DC P-60,L 36,E-4 HB-9.2,ESR 8/16, RBS -112, UR-20 CR-0.8
op no 53552	75	M	coolie	discharge	15 years	Rt	Rt-above medial malleolus	DM		ALCOHOLIC/SMOKER	YES	B/L SUPERFICIAL VEINS DILATATION ,BK,PERFORATOR I/C	TC-8800,DC-P-60,L-36,E-4,HB-12.2,ESR-18/32,RBS-90,UR-22,CR-0.8
op no : 85/2015	61	M	Security worker	pain	2 years	Rt	Rt-above medial malleolus	HT		NIL	YES	Rt-BK PERFORATOR I/C	TC-6200,DC P-70,L0 HB-12.2 ESR 8/16, RBS-120,UR-30,CR-0.8
op no :41456	55	F	house wife	pain	10 years	Rt	GAITER REGION			NIL	YES	AK,BK, PERFORATOR I/C	TC-5600,DC-P-54,L-38,E-8,HB-12.2GMS,ESR2/8,RBS-128 UREA-20,CREATININ
op no:9235	59	F	bhajji shop owner	Pain/discharge	10 years	Rt	Rt -above medial malleolus			NIL	yes	Rt-SFV junction I/C Grade II	TC-5800,DC-P72,L-28,E-2,Hb-12.2,ESR-8/16,RBS-120 urea-20,creatinine-0.8
op no:1805110	65	F	construction worker	pain/discharge	10 years	RT	Rt-above medial malleolus			NIL	YES	Normal arterial study Normal venous flow OF Lt-LL,Rt-AA,BK PER	TC-5200,DC-P-62,L-36,E-2,HB-9.8 ESR20/40,RBS 90,UR-25,CR-0.6
op no:1186110	43	M	supervisor in a market	pain	5 years	Rt	Rt-above medial malleolus		Recurrent attacks of DVT,Foam sceloro therapy done -3	ALCOHOLIC/SMOKER	NIL	Rt-SFJ AND PERFORATOR I/C	TC-5200, DC P-70,L-30,ESR 2/8,HB 14.2, RBS 90, UR-15,CR-0.6
op no:58020	70	M	coolie	pain/swelling	15 years	Rt	Rt-above lateral malleolus	SHT	RTA-20 years back, Plating done for R femur,Haemorrh	Alcoholic	Yes	Normal venous flowboth sides Rt-BK PERFORATOR I/C	TC-9700,DC-P72,L 26,HB-10.2,ESR 20/40,RBS-120,UR-28,CR-0.6
0p no:415/2015	43	M	watchman	pain and odour	3 years	Rt> Lt	B/L MEDIAL MALLEOLUS ULCERS	DM		SMOKER	YES	B/L SVJUNCTION INCOMPETENCE GRADE I B/L-BELOW KNEE PE	TC-8600,UR-18,CR-0.6,DC-P45,L-45,E-10,RBS-88,HB-12.1 ESR 2/8
opno:172895	43	M	farmer	pain and discharge	15 years	Rt	Rt-above medial malleolus		operated years back for varicose veins	Alcoholic	YES	Recanalised GSV,Dilated CFV/Popliteal Vein	TC-10200,DC-P-70,L-28,E-2,HB-14.2,ESR-2/8,RBS 98,UR-26,CR-1.0
op no :85324	65	F	household worker	pain	5 years	Lt	Lt-Above medial Malleolus	OBESE			YES	B/L,SFV JUNCTION I/C	TC-7800,DC P-68,L26,E-6,HB-10.2, ESR 2/8,RBS-118,UR-26,CR-0.6
opno:7659	63	F	cook	Pain	5 years	Rt	Rt-above medial malleolus				NIL	B/L AK,BK perforator I/C	TC-5400,DC-P72,L-28,HB-12.2,ESR 2/8,RBS-108,UR-26,CR-0.6
op no 838/12	47	M	Mechanic	pain/discharge	10 years	Lt	Lt-Above medial Malleolus			SMOKER	YES	Dilated SFV Junction I/C AK,BK,PERFORATOR I/C-LT	TC-9200,DC P-54,L-38,E-8 HB-16.2,ESR-4/8 RBS-98,UREA-20 CREATININE-0.6
OP NO 865/09	52	M	Farmer	PAIN	15 YEARS	Rt	Rt-above medial malleolus			ALCOHOLIC	NIL	B/L SFV JUNCTION I/C,BK,B/L PERFORATOR I/C	TC-5200,DC-P-64,L-36,,HB-15.8 ESR4/8,RBS -120,UR-16,CR-0.6
op no: 353/12	70	M	coolie	discharge	14 years	Lt	Behind Lt- medial malleolus			ALCOHOLIC/SMOKER	NIL	B/L SFV JUNCTION I/C,	TC-5600,DC-P-70,L-30,ESR2/8,HB-16.2 ,RBS-102,UR-20,CR-0.6
op no:1039/12	51	M	Mason	discharge	4 months	Lt	Lt-Above medial Malleolus				yes	B/L BK perforator I/C	TC-6200,DC-P68,L-26,E-6,HB-10.2,ESR4/8,RBS-118,UR-22,CR-0.6
op no 792/09	53	M	Mechanic	pain	5 years	Lt	Lt-Above medial Malleolus	OBESE		ALCOHOLIC/SMOKER	YES	LT-BK AA PERFORATORS I/C	TC-7200,DC-P-62,L-28,E-10,ESR-22/50,HB-10.2,RBS-98,UR-22,CR-0.9
op no: 14856	28	M	Autodriver	Pain/Discharge	5 years	Rt	Rt-medial malleolus extending to lateral malleolus	OBESE	Operated for perforators -2 years	Alcoholic	yes	Rt-SFV incompetence gradeII	TC-5200,P56,L-34,M-2,HB-15.2,ESR 2/8 ,RBS-94,UR-24,CR-0.8
op no:2060/12	42	F	Tailor	Pain	12 years	Rt	Multiple ulcer above Rt medial malleolus	Known case of RHD -MVR _20 years			yes	Rt-SFV incompetence gradeII,Below knee perforator incompeter	TC-6200,P-68,L-32 HB-10.2,ESR 10/24 ,RBS-82,UR-18,CR-0.8
op no:536/6	38	F	Tailor	Pain	2 years	Rt	Rt-medial malleolus above				yes		TC-6600,DC-P-54,L-44,E -2,HB-10.2 ESR-8/16,RBS-80,UR-20,CR-0.8
op no:28042	59	M	Security worker	Pain/Discharge	7 years	Lt	Above medial malleolus multiple	Dm	Operated 2 years SSG done	Alcoholic	nil	Lt-perforators incompetence Rt-LL SFJ incompetence grade II	TC-7200,DC-P68,L-32,HB-12 GR,UR-30,ESR-20/40,CR-0.8,
op no:28081	30	M	Tailor	Pain	2 years	Lt	Lt-Above medial malleolus		Operated for B/L varicose veins 7 years back	Alcoholic/smoker	Rt-LL dilate	Rt-SFJ incompetence	TC-7200,DC-P56,L-38,E-6 HB-14 GR,UR-20,ESR-8/16,CR-0.8,
op no: 2354/14	60	M	Coolie	Pain/Discharge	15 years	Rt	Lt-lateral malleolus			Alcoholic/smoker	nil	I/Cperforators at bB/A,BK,AK ,B/L grade ISF junction incompeter	TC-7200,DC-P76,L-26,Hb-15.1 GMs,ESR-4/8 ,RBS 82,UR-20,CR -0.8
op no: 26175	60	M	Autodriver	pain	15 years	Rt	Rt-above medial malleolus	Known case of RHD B/L varicose veins with Lt ulcer			yes	B/L GSV grade I Incompetence, Perforator incompetence B/L BK,	TC-5400,DCP-72,L 26 HB-15.1 GMS,ESR 10/24,RBS -140,UR-22,CR-0.8,
op no: 668/2015	60	M	Farmer	Pain/Discharge	15 years	Lt	Rt-medial malleolus extending to lateral malleolus	Dm	Operated Lt leg for varicosities : Herniorrhapy done 5 years back		nil	I/Cperforators at B/L A/A,BK,AK ,B/L grade ISF junction incompe	TC-8200,P60,L2,E8,12.1 ,2/8,RBS-172,UR-14,Cr-1.0
op no: 21888	70	M	Farmer	Pain	10 years	Lt	Multiple ulcer above Rt medial malleolus	HT/DM		SMOKER	yes	Lt-SFVJ incompetence GRADE I	TC-6800,DC-P54,L-42 ,E-4,HB-12.8,2/8/RBS-98,UR18,CR-0.8
op no;765/2015	52	M	Tailor	Pain/discharge	7 years	Rt	Rt-medial malleolus above		Operatedfor VARICOSE VEINS 4 years Rt side	SMOKER	YES	Rt-SF Junction incompetence gradeII	TC-5400,DC-P-56,L-44,ESR 4/8,HB-12.2,RBS-108,UR-20,CR-0.6
op no: 19002/12	61	M	Worker in a shop	PAIN	5years	Rt	Above medial malleolus multiple	DM/HT			NIL	Rt-SF Junction incompetence gradeII	TC-7800,DC-P-70,L-30,HB-12.1,ESR 2/8,RBS-108,UR-20,CR-0.6
OPNO:128675	60	M	FIRE OFFICER	pain	2months	Lt	Lt- back of medial malleolus			Alcoholic/SMOKER	YES	LT-AA,AK,BK,PERFORTORSI/C	TC-6200,DC-P-70,L30,HB-13.1 ESR-8/16,F-108,PP-120,UR-20,CR-0.6
op no: 78459	34	M	Supervisor at Uds	pain/Discharge	3 months	Lt	multiple ulcer above medial malleolus				yes	B/L multiple perfortar incompetence	TC-5800,DC-P-72,L28,HB-12.2, ESR 4/8,RBS-98,UR-26, CR-0.6
OP NO79856	67	M	Watchman	pain	8 years	Rt	Rt- above medial malleolus		DVT 2 years back,Known case of RHD ,MS,MR	Alcoholic/smoker	yes	Rt- Recalcitrant DVT	TC-6200,DC-P-60,L-32,E-8,HB-12.2, ESR 4/8,RBS-108,UR-27, CR-0.9
op no :1588	67	M	cook	pain/Discharge	20 years	Lt	Lt- above lateral malleolus				yes	Lt-SFV junction incompetence grade I BK<AK AA perforation I/C	TC-7200,DC-P-72,L-28,HB-10.2, ESR 4/8,RBS-108,UR-26, CR-0.6
op no;35789	70	F	watchman	pain/Discharge	15 years	Rt	Rt- above medial malleolus	OBESE			yes	Rt- SF junction incompetence grade II	TC-5400,DC-P-68,L-26,E-6,HB-6.8,ESR-20/40,RBS-83,UR-20, CR-0.8
op no :131589	74	M	ironman	pain/Discharge	20 years	Rt	Rt- above medial malleolus	Dm		Alcoholic/smoker	yes	Rt-SFV junction incompetence grade I	TC-7800,DC-P-62,L-36,E-2,RBS 80,UR-20,CR-0.9
op no :67586	65	M	cook	PAIN	5 YEARS	Rt	Rt- above medial malleolus				yes	Rt-sfV junction incompetence grade I,AK,BK,PERFORATOR INCO	TC-6800,DC-P 70,L-30,HB-12.2,ESR8/20
op no:4016	48	M	securityofficer	Pain	15 years	Rt	Rt- above medial malleolus	OBESE		Alcoholic	yes	Rt- BK,AA,AK PERFORATOR INCOMPETENCE RT- SFV JUNCTION	TC-6800,DC-P 61,L-37,E-2 HB-10.9,ESR 8/16
OP NO: 24675	67	M	Farmer	Pain/discharge	25YEARS	Lt	Lt-above medial Malleolus			SMOKER	YES	Lt-SFV junction incompetence grade II	TC-6200,DC-P-60,L-36,E-3,HB-11.2, ESR 4/8,RBS-98,UR-20 CR-0.9
op no:1996	46	M	Coolie	pain	15 years	Rt	Rt-above medial malleolus	CRF/DM			YES	B/L-AA,BK,MID THIGH CALF PERFORATORS I/C	TC-6200,DC-P-70,L30,HB-8.2, ESR-20/40,F-228,PP-308,UR-43,CR-1.6
op no: 42899	80	M	Ironman	discharge	10 years	Rt	Rt-above medial malleolus				yes	Rt-SF Junction incompetence gradeII,AA,BK,I/C	TC-7800,DC-P-56,L-44,HB-12.2,ESR-2/8,RBS-98,UR-22,CR-0.6

DGS	Aerobic-1	AEROBIC BACTERIA -2	ANAEROBIC BACTERIA	COLONY C	COLONY C	PEN	ERY	AK	CIP	COTRI	CX	PT	GM	CAZ	CTX	TETRA	IMP	VANCO	CLINDA	HLG
NO PUS CELLS ,few gpc in clusters	Staphylococcus aureus (MSSA)	NIL	NIL	$2.4 x10^4$		S	S	S	S	S	S		S			S		S	S	
NO PUS CELLS ,NO ORGANISM	Staphylococcus aureus (MSSA)			$2.4 x 10^2$		S	S	S	S	S	S		S			S		S	S	
Many pus cells,No organism	Staphylococcus aureus (MRSA)	NIL	NIL	$2.5 x 10^6$		R	R	S	R	R	R		S			R		S	S	
Few pus cells ,Many GNB seen	Proteus mirabilis ,	Enterococcus faecalis		$6.8 X10^6$	$9X10^5$	NT/S	NT/S	S/NT	S/S	S/NT	S/NT	S/NT	S/NT	S	S	S/NT	S/NT	NT/S		NT/S
Few pus cells ,few GNB seen	Staphylococcus aureus (MSSA),	Pseudomonas aeruginosa		$1.9 x10^7$	$5.6x10^5$	S/NT	S/NT	S/R	S/R	S/NT	S/S	NT/S	S/R	NT/S	NT	S/NT	NT/S	S/NT	S/NT	
few pus cells ,No organism	Proteus mirabilis		Peptostreptococcus anaerobicus	$3.6 X10^4$		NT	NT	S	S	S		S	S	S	S	S	S			
NO PUS CELLS ,FEW GPB SEEN	Diphtheriods			$8.5X10^4$		S	NT	NT	S	NT			NT		NT	NT		S		
FEW EPITHELIAL CELLS,NO PUS CELLS	Proteus mirabilis			$4.2 x10^5$		NT	NT	S	R	R	S	S	S	S	S	S	S			
FEW EPITHELIAL CELLS,many GNB SEEN	Proteus mirabilis		BACTIRIODIESFRAGILIS	$1.2 x 10^7$		NT	NT	S	S	S	S	S	S	S	S	R	S			
FEW PUS CELLS; NO ORGANISM	NO GROWTH																			
FEW PUS CELLS ,NO ORGANISM	Staphylococcus aureus (MRSA)			$3.4 X10^6$		R	R	S	S	R	R		S			S		S	S	
NO PUS CELLS NO ORGANISM	Acinetobacter baumanii		Peptostreptococcus anaerobicus	$1.2X 10^4$		NT	NT	S	S	S	S	S	S	S	S	S	S			
FEW PUS CELLS ,FEW GNB SEEN,	Proteus mirabilis ;	Enterococcus faecalis	Peptostreptococcus anaerobicus	$5.3x 10^4$	$1.4x 10^6$	NT/S	NT/S	S/NT	S/S	S/NT	S/NT	S/NT	S/NT	S/NT	S/NT	S/NT	S/NT	NT/S		NT/S
FEW PUS CELLS,many GNB SEEN	Pseudomonas aeruginosa	Escherichia coli	Peptostreptococcus anaerobicus	$1.1x10^6$	$2.5 x10^4$	NT	NT	S/S	S/R	NT/S	S/S	S/S	S/S	S/S	NT/S	NT/R	S/S			
NO PUS CELLS ,NO ORGANISM	Proteus mirabilis		Peptostreptococcus anaerobicus	$8.4X10^4$	$2.4 x10^3$	NT	NT	S	S	S	S	S	S	S	S	R	S	NT		
No Pus cells;FewGPC in clusters,FewGNB seen	Staphylococcus aureus (MSSA)	Escherichia coli (ESBL)		$2.7 x10^7$	$4.4 X 10^6$	S/NT	S/NT	S/S	S/S	S/S	S/S	NT/S	S/S	NT/R	NT/R	S/S	NT/S	S/NT	S/NT	
FEW PUS CELLS ,NO ORGANISM	S.epidermidis		Peptostreptococcus anaerobicus	$1.2X 10^4$		S	S	R	S	S	S		S			S		S	S	
NO PUS CELLS ,GPCS IN CLUSTER SEEN	S.epidermidis			$3.5 X10^7$		S	S	S	R	R	S		S			S		S	S	
NO PUS CELLS ,ManyGNB seen	Proteus mirabilis ;	Micrococci		$7.2 x 10^6$;	$1.2X10^2$	NT	NT	S	S	R	S	S	R	S	S	R	S			
NO PUS CELLS ,many GPCS IN CLUSTER SEEN	Staphylococcus aureus (MRSA)			$5 X 10^6$		R	R	S	R	S	R					S		S	S	
NO PUS CELLS NO ORGANISM	NO GROWTH																			
NO PUS CELLS NO ORGANISM	Micrococci																			
NO PUS CELLS NO ORGANISM	Staphylococcus aureus (MSSA)			$2 X10^2$		S	R	S	R	S	S	NT	S	NT	NT	S	NT	S	S	
NO PUS CELLS ,Few GNB seen	Proteus mirabilis			$3.4 X10^4$		NT	NT	S	R	S	S	S	S	S	S	S	S			
NO PUS CELLS NO ORGANISM	NO GROWTH																			
OCC Epithelial cells, No Pus Cells, No Organisms	Escherichia coli (ESBL)	Micrococci		$1.6X 10^7$	$2 X10^2$	NT	NT	S	S	S	S	S	S	R	R	S	S			
NO PUS CELLS ,NO ORGANISM	Staphylococcus aureus (MSSA)			$8.4 X10^3$		S	S	S	S	S	S		S			S		S	S	
NO PUS CELLS Few GPC in chains	GAS			$3.4 X 10^4$		S	S		S						S	S		S	S	
NO PUS CELLS ,MANY GPCS IN CLUSTER:MANY GNB SEEN	Pseudomonas aeruginosa;	Staphylococcus aureus (MSSA)		$5.9x10^4$	$1.1x10^5$	NT/S	NT/S	S/S	S/S	NT/S	S/S	S/NT	S/S	S/NT	NT	NT/S	S/NT	NT/S	NT/S	
NO PUS CELLS NO ORGANISM	Escherichia coli (ESBL)			$3X 10^7$		NT	NT	S	S	R	S	S	S	R	R	S	S			
few pus cells ,few gnb seen	Klebsiella pneumoniae			$4.2 x10^3$		NT	NT	S	S	S	S	S	S	S	S	S	S			
NO PUS CELLS ,NO ORGANISM	Staphylococcus aureus (MRSA)			$3.2 X 10^7$		R	R	S	S	R	R	NT	R	NT	NT	S		S	S	
NO PUS CELLS ,Few GNB seen	Pseudomonas aeruginosa;			$1.25 X10^6$		NT	NT	S	S	NT	S	S	S	S	NT	NT	S			
NO PUS CELLS ,NO ORGANISM	S.epidermidis			$3.4 X10^4$		S	S	S	R	S	S		S			S	S	S	S	
NO PUS CELLS ;MANY GNB SEEN	Pseudomonas aeruginosa;		Diphtheriods	$3.6 X 10^5$	$2 X10^2$	NT/S	NT	S/NT	S/S	NT	S/NT	S/NT	S/NT	S/NT	NT	NT	S/NT	NT/S		
NO PUS CELLS ,NO ORGANISM	Pseudomonas aeruginosa;			$1.4x10^2$		NT	NT	R	R	NT	S	S	R	S	NT	NT	S			
NO PUS CELLS,MANY GPB SEEN	Pseudomonas aeruginosa;			$3.4 X10^7$		NT	NT	S	S	NT	S	S	R	S	NT	NT	S			
NO PUS CELLS ,NO ORGANISM	Staphylococcus aureus (MSSA)			$5.2 X10^2$		S	S	S	S	R	S		R			S		S	S	
NO PUS CELLS,NO ORGANISM	Staphylococcus aureus (MSSA)			$1.2 X10^2$		S	S	S	R	S	S		R			R		S	S	
NO PUS CELLS ,NO ORGANISM	Micrococci			$1.4 x10^2$																
FEW PUS CELLS,NO ORGANISM	Staphylococcus aureus (MSSA)	Diphtheriods		$6.2 X10^4$	$3.4X 10^2$	S/S	S/NT	S/NT	S/S	S/NT	S/NT		S/NT			S/NT		S/S		
FEW PUS CELLS, FEW GNB SEEN	Pseudomonas aeruginosa;			$1.25 X10^5$		NT	NT	S	S	NT	S	S	R	S	NT	NT	S			
NO pus cells; Occ GNB seen	Klebsiella oxytoca			$5.4 x10^4$		NT	NT	R	S	S	S	S	R	S	S	R	S			
NO PUS CELLS,Many GPC in clusters	Staphylococcus aureus (MRSA)			$3.27 X10^7$		R	R	S	S	R	R	NT	S	NT	NT	S		S	S	
NO PUS CELLS,NO ORGANISM	Enterococcus faecalis			$2.4 x10^3$		S	S	NT	S	NT	NT	NT	NT	NT	NT	NT	NT	S		S
NOPUS CELLS ,NO ORGANISM	NO GROWTH																			
NO PUS CELLS ,NO ORGANISM	Proteus vulgaris			$1.2 X10^2$		NT	NT	S	S	S	S	S	S	S	S	S	S			
NO PUS CELLS ,NO ORGANISM	Pseudomonas aeruginosa;			$3.2X10^5$		NT	NT	S	S	NT	S	S	S	S	NT	NT	S			
FEW PUSCELLS ,FEW GPC IN CLUSTERS	Staphylococcus aureus (MSSA)	GAS		$4.2 X10^2$	$2.3 X10^2$	S/S	S/S	S/NT	S/S	S/NT	S/NT		S/NT		NT/S	S/S		S/S	S/S	
FEW GPC IN CLUSTERS SEEN,FEW PUS CELLS	S.epidermidis			$5.2 X10^5$		S	S	S	R	R	S		R			S		S	S	
Few Pus cells ,few GNB seen	Pseudomonas aeruginosa ;	diphtheriods		$6.6 x 10^3$	$1.4 X 10^4$	NT/S	NT	R/NT	S/S	NT	S/NT	S/NT	R/NT	S/NT	NT	NT	S/NT	NT/S		
NO PUS CELLS NO ORGANISM	Klebsiella oxytoca	Enterococcus faecalis		$3 x 10^4$	$1.2 x10^3$	NT/S	NT/R	S/NT	S/S	S/NT	S/NT	S/NT	S/NT	S/NT	S/NT	S/NT	S/NT	NT/S		S
NO PUS CELLS ,ManyGPC in clusters	Proteus vulgaris	Staphylococcus aureus(MSSA)		$1.2X10^3$	$3.4 X 10^5$	NT/S	NT/S	S/R	R/R	R/S	S/S	S/NT	S/R	S/NT	S/NT	S/S	S/NT	NT/S	NT/S	
NO PUS CELLS ,Many GNB seen	Pseudomonas aeruginosa	Escherichia coli (ESBL)		$1.4 X 10^5$	$3.5 X10^6$	NT	NT	S/S	S/S	NT/S	S/S	S/S	S/S	S/R	NT/R	NT/S	S/S			
Few Pus cells ,No organism	Staphylococcus aureus(MSSA)	Klebsiella oxytoca		$5.4 x 10^5$	1.2 x103	S/NT	R/NT	S/S	R/S	R/S	S/S	NT/S	S/S	NT/S	NT/S	R/S	NT/S	S/NT	S/NT	
FEW PUS CELLS ,MANY GNB MANY GPC IN PAIRS SEEN	Proteus vulgaris		Peptostreptococcus anaerobicus	$4.6 X10^4$		NT	NT	S	S	S	S	S	R	S	S	R	S			
FEW PUS CELLS ,FEW GNB seen, FEW GPC IN PAIRS	Pseudomonas aeruginosa,	Staphylococcus aureus (MSSA)		1 x 106,	1.2 X 106	NT/S	NT/S	S/S	R/R	NT/R	S/S	S/NT	S/S	S/NT	NT	NT/S	S/NT	NT/S	NT/S	
NO PUS CELLS NO ORGANISM	Staphylococcus aureus (MRSA)	Diphtheriods		$4.4 X 10^6$	$1.1 X 10^2$	R/S	R/NT	S/NT	S/S	S/NT	R/NT	NT	S/NT	NT	NT	R/NT	NT	S/S	S/NT	
NO PUS CELLS FEW GNB SEEN	Pseudomonas aeruginosa	S.epidermidis		$1.4 X 10^3$	$2.8 x10^3$	NT/S	NT/S	R/S	R/S	NT/S	S/S	S/NT	R/R	S/NT	NT	NT/S	S/NT	NT/S	NT/S	
fewpuscells,few gnb seen	Proteus vulgaris	Escherichia coli		$5.4 X 10^4$	$2.6 X10^3$	NT	NT	S/S	R/S	S/R	S/S	S/S	S/S	S/S	S/S	S/S	S/S			
NO PUS CELLS,NO ORGANISM	NO GROWTH																			
few pus cells ,No organism	Escherichia coli	S.epidermidis		$3.2X10^4$	$5.4X10^5$	NT/S	NT/S	S/S	S/S	R/R	S/S	S/NT	R/S	S/NT	S/NT	S/NT	S/NT	NT/S	NT/S	
NO PUS CELL, NO ORGANISM	Staphylococcus aureus (MSSA)			$2.9 X 10^2$		S	S	R	R	S	S		R			S		S	S	
NO PUS CELL, NO ORGANISM	Staphylococcus aureus (MSSA)			$1.3 X10^4$		S	S	S	S	S	S		S			S		S	S	
NO PUS CELL, FEW GNB SEEN	Klebsiella pneumonia ;	Peptostreptococcus anaerobicus		$5.2X 10^5$		NT	NT	R	R	S	S	S	R	S	S	S	S			
NO PUS CELL, many GNB seen	Escherichia coli (ESBL)			$3.4 X10^6$		NT	NT	R	S	R	S	S	R	R	R	R	S			
FEW PUS CELLS ,few GPCS IN CLUSTERS seen	Staphylococcus aureus (MRSA)			$4.2 X10^6$		R	S	S	R	R	R		S			S		S	S	
FEW PUS CELLS ,NO ORGANISM	NO GROWTH																			
NO PUS CELL, NO ORGANISM	Proteus mirabilis	Micrococci		$1.7 X 10^2$	$2.6 X10^3$	NT	NT	R	S	R	S	S	R	S	S	S	S			
FEW PUS CELLS,NO ORGANISM	Escherichia coli	Staphylococcus aureus(MSSA)		$3.4 X10^3$	$1.1 X 10^2$	NT/S	NT/S	S/S	S/R	S/S	S/S	R/NT	S/S	S/NT	S/NT	S/S	S	S/S	NT/S	
FEW PUS CELLS, FEW GNB SEEN	Proteus mirabilis	Staphylococcus aureus (MSSA)		$1.6 X10^3$	$5.3 X10^3$	NT/S	NT/S	S/S	S/R	S/R	S/S	S/NT	S/S	S/NT	S/NT	S/S	S/NT	NT/S	NT/S	
NO PUS CELLS,NO ORGANISM	klebsiella Oxytoca ESBL			$3.5 X 10^6$		NT	NT	S	R	R	S	R	S	R	R	S	S			

op no:83096	65	M	cook	Pain/discharge	15 years	Rt	Rt-above medial malleolus,extending into dorsum			Alcoholic	NIL	Rt-SF Junction incompetence gradeII,	TC-5400,DC-P45,L-50,E-4,M-1
op no:9384/15	52	M	watchman	pain	8 years	Rt	Rt-above medial malleolus			Alcoholic/smoker	Rt-dilated	Rt-SF Junction incompetence gradeII,AA,BK,I/C	TC-5800,DC-P-62,L-6,E-2,HB-15.2 ESR 4/8,RBS-120,UR-20,CR-0.6
op no:48037	37	M	cook	Pain/discharge	3 years	Lt	Lt-above medial Malleolus	OBESE		ALCOHOLIC/SMOKER	B/L Varicos	B/L LL WITH MULTIPLE I/C PERFORATORS GRADE II SF JUNC I/C	TC-4100,DC-P-50,L-42,E-8,HB-14.1,ESR-6/12,RBS-118,UR-19,CR-0.8
OPNO 49800	80	M	Watchman	Foul smell/dischar	18 years	Lt	Lt-above medial malleolus			Alcoholic/Smoker	YES	Lt-PARTIAL DVT	TC-5800,DC-P-62,L-30,E-8HB-10.8,ESR-66,RBS-118,UR-20,CR-0.6
op no;445/09	72	M	Farmer	Pain	3 years	Rt	Rt-above medial malleolus				YES	Rt-BK,Mid calf Perforators I/C	TC-5400,DC-P-54,L-46,HB-12.8,ESR 2/8,RBS-98,UR-20,CR-0.6
OP NO:185/08	38	M	Saloon shop worker	Increased pain and	10 years	Rt	Rt-above medial malleolus		Skin grafting done 3 years back	NIL	NIL	Rt-GSV dilated and torsurous, Below knee perforator and Slow fl	TC-8400,DC-P60,L32,E7,HB-11.9,ESR-10/35,RBS-95,UREA-30,CR-1.1
OP NO:2071/14	78	M	watchman	discharge	7 YEARS	Lt	Lt-above lateral Malleolus		Operated for 4 years for varicose veins	ALCOHOLIC/SMOKER	NIL	Lt-SFV Junction incompetence grade -I,Multiple perforators Inco	TC-5400,DC-P-68,L-26,E-6,HB-7.9,ESR-20/40,RBS-83,UR-20, CR-0.8
OP NO: 05961	39	F	FARMER	discharge	5 years	Lt	Lt- above medial malleolus	obese			yes	B/E SFV JUNCTION INCOMPETENCE GRADE-1 B/L BK,AA with lat	TC-5800,DC-P62,L30,E8,HB-7.7,ESR-66,RBS-83,UREA-21,CR-0.6
OPNO186956	23	M	Leather factory supervisor	discharge	1 year	lt	Lt- above medial malleolus			NIL	NIL	Lt-Femoral DVT with B/K perforator incompetence,GSV -NORMA	TC-6800,DC-P78,L15,E7,RBS-65,UREA-23,CR-1.0ESR-20,HB-10.5
op no:138219	72	M	TEA MASTER	Pain	20 YEARS	Rt	Rt-above medial malleolus	inguinal he	GSV strapping done -5years back	ALCOHOLIC/SMOKER	NIL	B/L BK,AK,PERFORATOR INCOMPETENCE	TC-5200,P56,L-34,M-2,HB-15.2,ESR 2/8 ,RBS-94,UR-24,CR-0.8
OP NO 192456	28	M	Tiles worker	pain	3years	Rt	Rt-above medial malleolus	obese	OPERATED FOR VARICOSE VEINS - 3YEARS	SMOKER	yes	B/L PERFORATOR INCOMPETENCE	TC-4800,DC-P-64,L32,E4 HB-15.2,ESR -8/16,RBS-98,UR-22,CR-0.8
IP NO 56648	60	M	UNEMPLOYED	PAIN AND FEVER	10 years	LT	Lt-lateral malleolus			ALCOHOLIC	YES	LT-ILEOFEMORAL DVT	TC-15800,DC-P94,L3,M3,HB-8.2 RBS-128,UREA-40,CR-2.5
OP NO 190141	32	M	Security worker	discharge	3YEARS	RT	Rt-above medial malleolus			NIL	YES	RT-Grossly dilated torturous vein found in thigh and leg AA inco	TC-5400,DC-P72,L-28,HB-12.2,ESR 2/8,RBS-108,UR-26,CR-0.6
OP NO 44644	47	M	Tea stall worker	Pus/discharge/Ma	2 years	Rt	Rt-above medial malleolus			NIL	YES	Grade II SF Junction Incompetence with R AA perforator R latera	TC-7000,DC-P80,L15,E,ESR24/HR MM,HB 16.8 RBS 66 UREA 4,CR-1.5
op no4932/12	70	M	retired pwd worker	swelling	15 years	Rt	Rt-above medial malleolus	DM		ALCOHOLIC	YES	B/L R- SF Junction grade-II, L-Grade-I B/L Mid calf perforator Inc	TC-10200,DC-P-70,L-28,E-2,HB-14.2,ESR-2/8,RBS 98,UR-26,CR-1.0
OP NO 1224/2015	49	M	Farmer	swelling/pain	2 yeasr	Rt	Rt-above medial malleolus			NIL	YES	Rt-Cfv -recanalised vein,Dilated Femoro popliteal vein SF Junctio	TC-6000,DC-P47,L40,E-10,M-3,HB-12.5,UREA-22, CR-1.1
OP NO 1762/14	65	M	TAILOR	discharge	3 years	Lt	Lt- above medial malleolus	DM/ CABG -5 YEARSBACK		NIL	YES	Lt-SFV Junction incompetence grade -I	TC-8000,DC P70,L0,HB-10.2 ESR-8/16,R 98, UR-, CR-0.9
OP NO 1689/15	50	F	TAILOR	PAIN	2 YEARS	RT	RT-lateral malleolus	OPERATED FOR IVC THROMBOSIS -5 YEARS BACK ON ANTICOAGU		NIL	YES	B/L GSV grade I Incompetence, Perforator incompetence B/L BK,	TC-9300,DC-P058,L34,E8,HB-9.8,RBS-120,UEA-24,CR-1.2
OP NO 773/10	57	M	COOK	pain and discharge	15 years	Lt	Lt- above medial malleolus				YES	Lt SFJI/C;AA PERFORATOR I/C	TC-8700,DCP73,L22,E5,HB-10.9,RBS-66,UREA-18,CR-1,1
OP NO 1965/15	27	M	Sales representative	Pain	3years	Rt	Rt-above medial malleolus				yes	Rt-recanalised GSV (Dilated) Fibrosis extending from ankle to SF	TC-4100,DC-P-50,L-42,E-8,HB-14.1,ESR-6/12,RBS-118,UR-19,CR-0.8
OP NO 19265	60	F	COOK	pain /discharge	10 years	Rt	RT-above medial malleolus	DM			yes	B/L SFV JUNCTION INCOMPETENCE	TC-5800,DC-P-62,L-30,E-8HB-10.8,ESR-66,RBS-118,UR-20,CR-0.6
OP NO 1076/12	40	M	XEROX SHOP OWNER	PAIN	6 YEARS	RT	RT-lateral malleolus-MULTIPLE	OBESE	SSG -6 YEARS	NIL	NIL	RT-AA PERFORATOR INCOMPETENCE	TC-7600,DC-P76,L24
OP NO 1267/15	56	M	watchman	discharge	20 years	LT	LT- lateral malleolus				YES	LT-AA,PERFORTORSI/C	TC-5200,DC-P72,L26,E2,HB-15.2,UREA-15,CR-0.7
OP NO 1489/15	63	F	Tailor	discharge	15 YEARS	LT	Lt-Above medial Malleolus	DM			YES	LT-Partially recanalised popliteal vein	TC-6900,DC-P73,L-26,E-1, HB 8.8,UREA-20,CR-1.1
OP NO6725	52	M	watchman	discharge	8 YEARS	LT	Lt-Above medial Malleolus	KNOWN CASE OF RHD -MVR _12 years B/L VARICOSE VEINS-10 YEARS			YES	Lt-SFV Junction incompetence grade -I,AA I/C	TC-68,HB-12.2 ,DC-P 47,L42,E 11,UREA-23,CR-0.8
OP NO 7269/14	44	M	TAILOR	PAIN	5 YEARS	RT	Rt-above medial malleolus			ALCOHOLIC	YES	Rt- SF junction incompetence grade II	TC-5600,DC-P-70,L-0,HB-15.0GMS,ESR2/8,RBS-128 UREA-30,CREATININE0.8
OP NO 246/09	55	M	UN EMPLOYED	DISCHARGE	1O YEARS	LT	Lt- above medial malleolus			ALCOHOLIC	YES	Lt-SFV Junction incompetence grade -I	TC-8300,DC-P82,L16,E2
IP NO 15472	67	M	WATCHMAN	DISCHARGE	5 YEARS	LT	LT-MULTIPLE ULCERS OVER THE lateral malleolus	DM/HT		SMOKER	YES	Lt-SFV Junction incompetence grade -I,Multiple perforators Inco	TC-5400,DC-P56,L36,E8,RBS-108,UREA-22,CR-1.1

GPC IN PAIRS AND SHORT CHAINS, NO PUS CELLS	Staphylococcus aureus (MRSA)	Enterococcus faecalis		$4.6 X 10^6$	$1.3 X 10^3$	R/S	S/R	S/NT	S/S	R/NT	R/NT	NT	S/NT	NT	NT	S/NT	NT	S/S		NT/S
NO PUS CELLS ,NO ORGANISM	Staphylococcus aureus (MSSA)	Acinetobacter baumanii		$2.6 X10^3$	$2.4 X10^4$	S/NT	R/NT	S/S	S/S	S/S	S/S	NT/S	S/S	NT/S	NT/S	S/S	NT/S	S/NT		
Few Pus CELLS, many GNB seen	Escherichia coli	Micrococci		$1.2 X10^7$	$4.3 X10^2$	NT	NT	R	R	S	S	S	R	S	S	S	S			
NO PUS CELLS,NO ORGANISM	Proteus vulgaris	S.epidermidis		$5.4X 10^5$	$2.8 x10^3$	NT/S	NT/S	S/R	S/S	S/S	S/S	S/NT	S/R	S/NT	S/NT	S/R	S	NT/S	NT/S	
NO PUS CELLS,Many GNB seen ,Few GPC in clusters seen	Staphylococcus aureus (MSSA)		Pseudomonas aeruginosa	$4.3 X10^4$	$2.7 X10^5$	S/NT	S/NT	S/S	R/R	R/NT	S/S	NT/S	S/R	NT/S	NT	S/NT	NT/S	S/NT	S/NT	
NO PUS CELLS ,NO ORGANISM	Enterococcus faecalis			$5.2 X10^4$		S	R	NT	S	NT	NT	NT	NT	NT	NT	NT	NT	S	NT	S
few pus cells and few gnb seen	Escherichia coli		Peptostreptococcus anaerobicus	$4.6 X10^2$		NT	NT	S	S	R	S	S	R	S	S	S	S			
NO PUS CELLS,MANY GPB SEEN	DIPHTHERIODS			$2.4 X 10^3$		S	NT	NT	S	NT	NT	NT				NT		S		
Many Pus cell seen,Many GPC in clusters	Staphylococcus aureus (MSSA)			$3.8 X10^7$		S	R	R	R	R	S		R			R		S	S	
NO PUS CELLS NO ORGANISM	NO GROWTH																			
NO PUS CELLS ,FEW GPC IN CLUSTERS	Staphylococcus aureus (MRSA)			$4. 3 X10^6$		R	S	S	S	R	R	NT	S	NT	NT	S		S	S	
Many pus cells,MANY GNB SEEN	Escherichia coli (ESBL)	S.epidermidis	Peptostreptococcus anaerobicus	$5.3 x10^6$	$2.4 x10^4$	NT/S	NT/S	S/S	S/S	R/S	S/S	S/NT	S/S	R/NT	R/NT	S/S	S	NT/S	NT/S	
Occ pus cells ,Many GPC in clusters	Staphylococcus aureus (MRSA)			$1.24 x10^7$		R	R	R	R	R	R	NT	R	NT	NT	R		S	S	
Many pus cells,MANY GNB SEEN	Escherichia coli	Enterococcus faecium		$2.5 x 10^7$	$3.1 x 10^2$	NT/S	NT/S	S/NT	S/R	S/NT	S/NT	S/NT	S/NT	S/NT	S/NT	S/NT	S/NT	NT/S		NT/S
Many pus cells,MANY GNB SEEN	Pseudomonas aeruginosa	Staphylococcus aureus (MSSA)		$4.3 x10^6$	$2.3 x10^5$	NT/S	NT/S	S/S	S/S	NT/S	S/S	S/NT	S/S	S/NT	NT	NT/S	S/NT	NT/S	NT/S	
NO PUS CELL FEW GPCS IN CLUSTERS SEEN	Staphylococcus aureus (MRSA)			$3.2 X 10^7$		R	S	S	S	S	R		S			S		S	S	
FEW PUS CELLS,FEW GNB SEEN, FEW GPC IN PAIRS SEEN	Klebsiella pneumoniae		Peptostreptococcus anaerobicus	$4.3x 10^4$		NT	NT	R	S	S	S	S	R	S	S	S	S			S
FEW PUS CELLS, NO ORGANISM	Escherichia coli	Enterococcus faecalis		$5.4 x 10^4$	$3.1 x 10^4$	NT/S	NT/S	S/NT	S/S	S/NT	S/NT	S/NT	S/NT	S/NT	S/NT	S/NT	S/NT	NT/S		NT/S
NO PUS CELL ;NO ORGANISM	NO GROWTH																			
no pus cells;FEW GPCS IN CHAINS AND PAIRS	GAS			$5.2 X10^4$		S	S	NT	S	S		NT	NT	NT	S	S		S	S	
no pus cells ;no organism	Staphylococcus aureus (MRSA)			$2.6 X10^6$		R	R	R	R	R	R	NT	R	NT	NT	R		S	S	
no pus cells ;no organism	s.epidermidis		BACTIRIODIESFRAGILIS	$3.5 x 10^4$		S	S	S	S	S	S		S			S		S	S	
few pus cells ,few GPCS IN CLUSTERS seen	Staphylococcus aureus (MSSA)			$1.2 X 10^2$		S	S	S	S	S	S					R		S	S	
FEW PUS CELLS;MANY GNB SEEN	Klebsiella oxytoca		Peptostreptococcus anaerobicus	$1.3x 10^6$		NT	NT	R	R	R	S	S	R	S	S	R	S			
NO PUS CELLS FEW GPC IN CLUSTERS	Staphylococcus aureus (MRSA)			$4.5 X 10^6$		R	R	R	S	R	R		R			R		S	S	
no pus cells ;no organism	Staphylococcus aureus (MSSA)			$2.6 X10^3$		S	S	S	R	R	S		S			S		S	S	
NO PUS CELLS NO ORGANISM	NO GROWTH																			
FEW PUS CELLS SEEN;FEW GNB SEEN	Proteus vulgaris	Enterococcus faecalis		$4.3 x10^6$	$5.5 x 10^6$	NT/S	NT/S	S/NT	S/S	S/NT	S/NT	S/NT	S/NT	S/NT	S/NT	S/NT	S/NT	NT/S		NT/S

KEY TO MASTER CHART

M-Male,F-Female,DM-Diabetes mellitus,HT-Hypertension,TC-Total count,DC-Differential count ,OP-outpatient,IP-Inpatient

R-Resistant, S-Sensitive NT- Not tested

*Clindamycin sensitivity was reported after testing with Inducible D test according to the CLSI guidelines.

** Sensitivity to Diphtheroids were interpreted according to British Society ofAntimicrobial Chemotherapy guidelines.

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